Computational and Molecular Analyses of HIV Epidemiology in Switzerland: From Quantifying Transmission and Coinfections to Behavioral Determinants of Health

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SUMMARY AND OUTLINE

To date, with one exception, HIV still cannot be cured. This emphasizes the crucial importance of diagnosis and treatment, on one hand, and prevention on the other. In this thesis, we approached HIV prevention from multiple angles. After a general introduction into HIV epidemiology and biology we begin by asking the question: How fast HIV is transmitted in Switzerland? In this work (chapter I), we showed that HIV in Switzerland is being transmitted very rapidly, with 43% of all transmissions occurring within the first year of the infection. Since many of the infected individuals are still not aware of their HIV positive status during the first year of infection, this emphasizes the importance of early diagnosis and demonstrates that the HIV epidemic in Switzerland cannot be contained only by treating all the diagnosed HIV positive individuals and that additional tools are needed. In this regard, Pre-Exposure-Prophylaxis (PreP) is currently the most promising one.

While PreP is currently in its infancy in Switzerland, prevention of HIV with antiretroviral therapy after possible exposure to the virus (Post-Exposure-Prophylaxis (PEP)) is available since almost two decades. What was not clear, however, is how accurately PEP is prescribed. In chapter II we investigated whether PEP is prescribed in situations that correspond to the highest risk of infection and withheld in situations that had no or a minute risk. At large, the answer is affirmative. We found that despite the hectic environment of the emergency room and the fact that most of the decisions were not made by infectious diseases specialists, the decisions accuracy rate stood at around 80%. Naturally, we also identified situations in which decision-making can still be improved and suggested ways on how to do so, for example by trying to reach out to the source partner.

In chapter III, we proceed with prevention, but this time we investigate whether hidden patterns recovered from Big Data can facilitate prevention and generate epidemiologically relevant insights. We used more than three decades of Swiss HIV cohort data with more than 400,000 clinical visits to show that some stable HIV-infected partnerships can be found in

cohort studies just because the patients frequently attend the clinic visits together. Using the clinical visits dates, and after extensive validation, we could detect twelve transmission pairs of a mixed ethnicity with a large median age gap. These patients harbored HIV-1 of predominantly non-B subtypes, which suggests that those infections were imported into Switzerland.

While the incidence of HIV among Men-who-have-sex-with-Men (MSM) in Switzerland is relatively stable, the incidence among injecting-drug-users (IDUs) has been almost eliminated. This is despite the fact that Switzerland had one of the largest open-drug-scenes in Europe during the early 1990s. This phenomenal success in terms of HIV prevention, is often attributed to the implemented harm reduction package (needle and syringe exchange and opioid substitution therapy). To examine that, we performed in chapter IV a quantitative evaluation of the cumulative impact of the implemented harm reduction measures. By combining a mathematical model with the unique data from the Swiss HIV Cohort Study (SHCS), the SHCS drug-resistance sequence database, national epidemiological data and data from previous works, we demonstrated that overall, harm reduction prevented around 15,000 HIV cases, 1980-2015. In addition, using a phylogenetic analysis, we demonstrated that the benefits of harm reduction extend beyond the population of injecting-drug-users, with around 2,500 averted spill-over infections to the general population.

From HIV prevention among injecting-drug-users we proceed with the problem of HIV and coinfections. Some subgroups of HIV positive individuals often maintain high levels of sexually risky behavior even after being infected with HIV. This exposes them to other sexually-transmitted-infections (STIs), like Syphilis, Gonorrhea and Chlamydia. The rates of STIs in the HIV positive population has been increasing over the last 10 years, which requires more studies that assist in optimizing screening and prevention strategies. In chapter 5, we systematically describe the characteristics and risk factors of individuals with a sexually transmitted infection (STI) in a well-characterized cohort of mainly men-who-have-sex-with-

men (MSM) with a prior diagnosis of primary HIV-1 infection (Zürich Primary HIV Infection study). We found a very high STI prevalence (33%), mostly asymptomatic at presentation, and identified three independent factors associated with a positive STI screen: i) anal intercourse, ii) reporting condomless sex, and iii) reporting any recent recreational drug use. These results highlight the importance of a more frequent (ideally 3-monthly) screening in high-risk populations as identified in this study, and demonstrate the potential of relying on self-reported sexual risk behavior and drug use data for screening prioritization.

Finally, from the prevention of HIV infection and other STIs, we move on to prevention of chronic illness, and specifically atherosclerosis in aging people living with HIV. This is a pertinent question since nowadays HIV positive individuals on successful antiretroviral therapy exhibit a life-expectancy that lags only few years behind the HIV negative population. However, since some HIV drugs have a deleterious effect on the lipids profile, and in light of the fact that some antiretroviral drugs interact with statins, there is a benefit also in the examination of life-style variables that might assist in preventing or reversing atherosclerosis. In chapter VI, we conclude this thesis with an analysis that examines the association between dietary habits, physical activity and total cholesterol in aging people living with HIV.

ZUSAMMENFASSUNG

Im Jahr 2016 übertraf die Anzahl der HIV Fälle in der europäischen Region der WHO erstmals die 2 Millionen Schranke. Gemäß des aktuellsten WHO/ECDC Reportes wurden im Jahr 2015 in den 50 Ländern der europäischen WHO Region 153.407 neue HIV Fälle diagnostiziert. Die epidemiologischen Einflussfaktoren unterscheiden sich jedoch nach wie vor im Westen und Osten Europas. In Westeuropa treiben hauptsächlich Männer, die Sex mit Männern haben (MSM), die Epidemie voran. In dieser Arbeit konnten wir vor allem den Einfluss von Transmissionen von Schweizer MSM während kürzlicher HIV Infektion und nach Unterbrechung der Behandlung genauer untersuchen. Des weiteren wurde im Rahmen einer Studie kritisch untersucht und charakterisiert, inwiefern die Verschreibung von Postexpositionsprophylaxe, welche vor allem von MSM gebraucht wird, die zukünftige HIV Prävention verbessern kann. Im Kontrast zu Westeuropa steht Osteuropa, wo Personen die Drogen injizieren die größte Risikogruppe darstellen. Erstaunlicherweise ist die Anzahl der AIDS Fälle in dieser Region in den letzten 10 Jahren um 80% angestiegen. Da die Schweiz in den 1980er Jahren eine Epidemie von Opium-Abhängigen durchmachen musste, könnten daraus wertvolle Erkenntnisse gewonnen werden, welche heute potenziell relevant für die HIV Prävention in Osteuropa sein könnten. Insbesondere wurde die essenzielle Rolle der Schadensverminderung beleuchtet, welche sowohl eine Eindämmung der Epidemie unter Personen, die Drogen injizieren, aber auch in der allgemeinen Bevölkerung erzielt hat. Des weiteren konzentriert sich diese Arbeit jedoch nicht nur auf die Prävention von neuen HIV Infektionen, sondern auch auf die Verbesserung der Gesundheit von Personen, die bereits mit HIV leben müssen. Dies wurde auf zwei Ebenen bearbeitet: (i) Wir haben eine hohe Rate an asymptomatischen sexuell übertragbaren Infektionen (STIs) gezeigt, sowie eine erhöhte Risikobereitschaft im Sexualverhalten von Patienten, welche während der kürzlichen Phase der HIV Infektion diagnostiziert wurden. Außerdem wurden epidemiologische Risikofaktoren für STIs identifiziert und empfohlen, dass intensivere Screenings durchgeführt werden sollten. (ii)

Wir haben die Ernährungs- und Bewegungsgewohnheiten von alternden HIV-positiven Patienten untersucht und die Ergebnisse mit den totalen Cholesterinwerten korreliert, in der Absicht die Prävention von koronaren Herzerkrankungen zu erleichtern.

Zusammenfassend haben wir in dieser These beabsichtigt zu verschiedenen Bereichen der HIV Prävention und klinischen Praxis beizutragen, in der Hoffnung sowohl Präventionsanstrengungen als auch Behandlungen zu verbessern.

References (Zusammenfassung)

1. HIV cases reach over 2 million for the first time in Europe [Internet]. 2016 [cited 2017 Sep 4]. Available from: http://www.euro.who.int/en/media-centre/sections/press-releases/2016/11/hiv-cases-reach-over-2-million-for-the-first-time-in-europe
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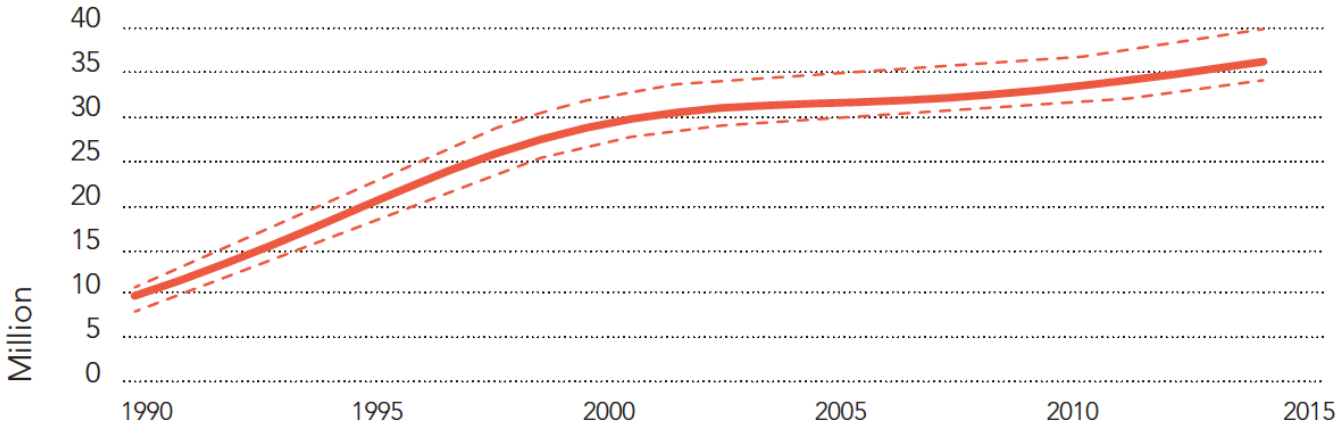
The global burden of HIV

According to the latest UNAIDS report, currently 36.7 million people are living with Human Immunodeficiency Virus (HIV) worldwide (Figure 1)[1]. In 2015 alone, there were 2.1 million new infections and 1.1 million Acquired Immune Deficiency Syndrome (AIDS) related-deaths.

Figure 1. The global number of people living with HIV by year. Source: UNAIDS [1]. Dashed lines represent uncertainty interval.



Most people with HIV live in Africa, with 19 million in Eastern and Southern Africa, and 6.5 million in the Western and Central parts. An estimated number of 2.4 million HIV infected live in Western and central Europe and North America altogether (Figure 2).

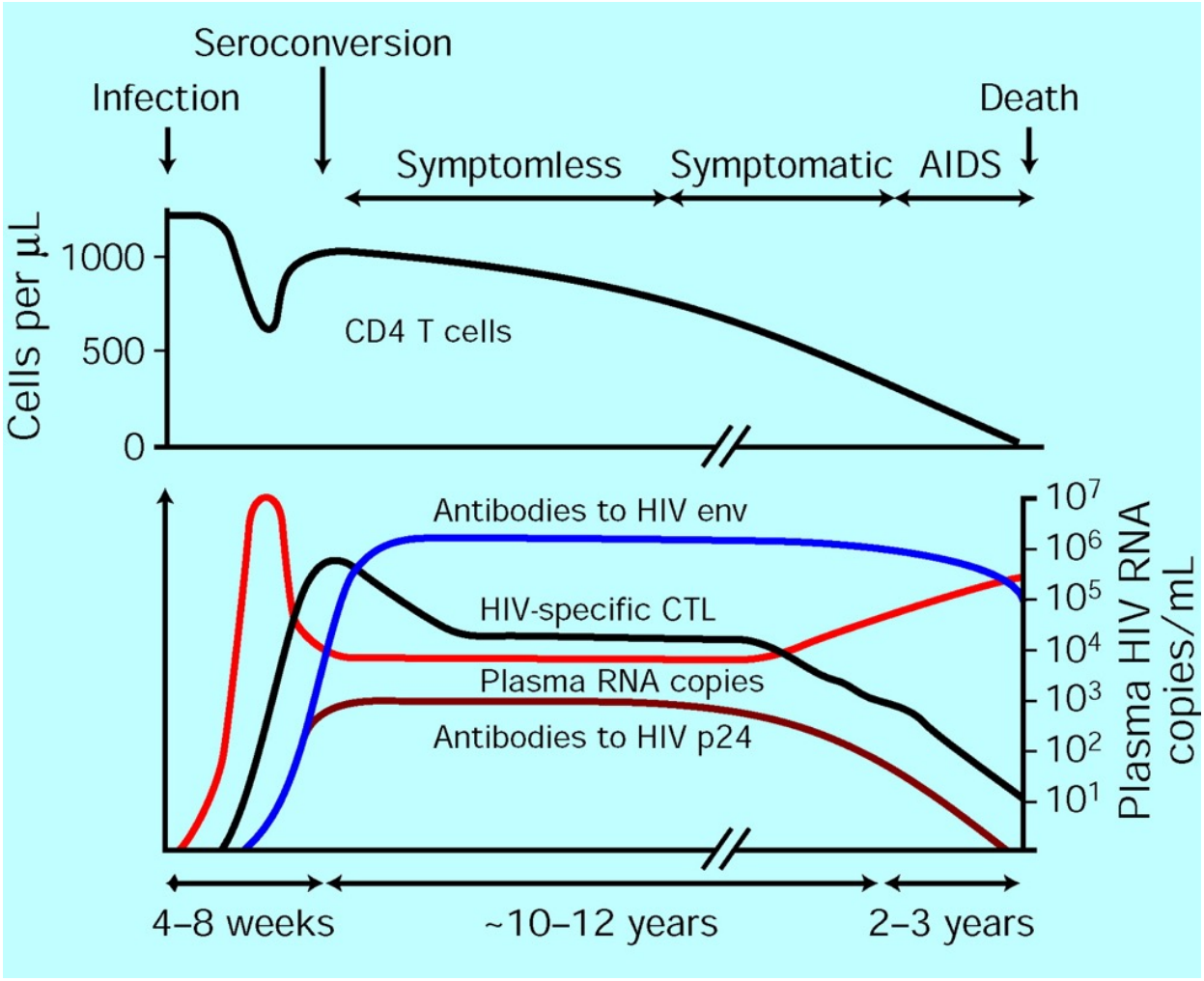
Figure 2. Adults and children estimated to be living with HIV (2015), by geographical region. Source: UNAIDS [1].

Disease progression

HIV infects CD4 T cells and if untreated gradually causes immunodeficiency and subsequently death from AIDS related cancers or opportunistic infections[2,3]. Except for a first brief episode during the acute phase[4] which is defined up to three months since seroconversion, the infection can be asymptomatic for years, until the AIDS stage is reached. The latter is characterized by severe immunodeficiency that leads to life-threatening opportunistic infections like *Pneumocystis pneumonia* [5] and fatal cancers like Kaposi Sarcoma[6].

The viral load temporally peaks within few weeks after transmission and within 3 to 6 months reaches a set-point value which is relatively stable for a few years and can predict both disease progression and the risk of transmission[7] (Figure 3).

Figure 3. Natural course of untreated HIV infection, source:[2]



Host and viral factors

Both host and viral factors were shown to shape the severity of the clinical course of HIV infection. From the host perspective, younger age at infection and carriage of two different Human Leukocyte Antigen (HLA) B alleles increase host infection tolerance and are associated with a slower diseases progression. Even more important is the resistance gene beta-chemokine receptor 5 (CCR5). This receptor serves as the main docking point for HIV upon entrance into the CD4 cell[8,9]. A 32−nucleotide deletion (delta-32) in this gene was shown to confer resistance to the establishment of HIV infection in homozygous individuals[10,11]. More on the host side, Rusert and Kouyos *et al.* showed that individuals of black ethnicity have higher post-infection induction rates of Broadly neutralizing antibodies (bnAbs) i.e. antibodies that are capable of neutralizing several viral strains, as compared to whites[12].

On the virus side, viral genetics was shown to explain around one-third of the set-point viral load[13–16] and there is evidence that the rate of the decline of the CD4-cell counts in the recipient and the viral-load independent pathogenicity ("per pathogen pathogenicity") are also partly determined by the viral genotype[14,17].

The origins of HIV

HIV is a Retrovirus, that belongs to the Lentivirus genus. It is closely phylogenetically related to Simian-Immunodeficiency-Virus (SIV) and studies have shown that HIV emerged from several cross-species transmission events from non-human primates (Figure 4)[18]. Accordingly, the HIV virus is classified in to two types HIV-1 and HIV-2, the latter was a result of a cross-species transmission from Sooty mangabeys, and has a reduced virulence as compared to HIV-1[19]. HIV-1, which constitutes the clear majority of infections worldwide, is further subdivided into four phylogenetic lineages, M N O and P. HIV-1 Group M, which originated from chimpanzees (Figure 5), is the most prevalent group, and the main causative agent of the HIV pandemic [20]. This group is further subdivided into subtypes and sub-subtypes, designated as A1, A2, A3, A4, B, C, D, F1, F2, G, H, J, and K, in addition to many circulating or unique recombinant forms. The various subtypes can differ by around 35% in the envelope (Env) glycoproteins of the virus [21,22,23]. The genetic variation within subtypes is lower an can range between 15% to 20% in Env [22]. Subtype B is the predominant subtype in Europe and North America. The strong geographic segregation of subtypes makes them useful for epidemiological surveillance and for detection of HIV introductions from other countries[24]. However, subtypes might also provide clinical insights as there is evidence, that there is heterogeneity in disease progression between subtypes [25,26]. For example, even after adjusting for a set-point viral load, HIV-1 subtype D was associated with a faster disease progression as compared to subtype A[27].

Figure 4. The Origins of human AIDS viruses. Source:[20].

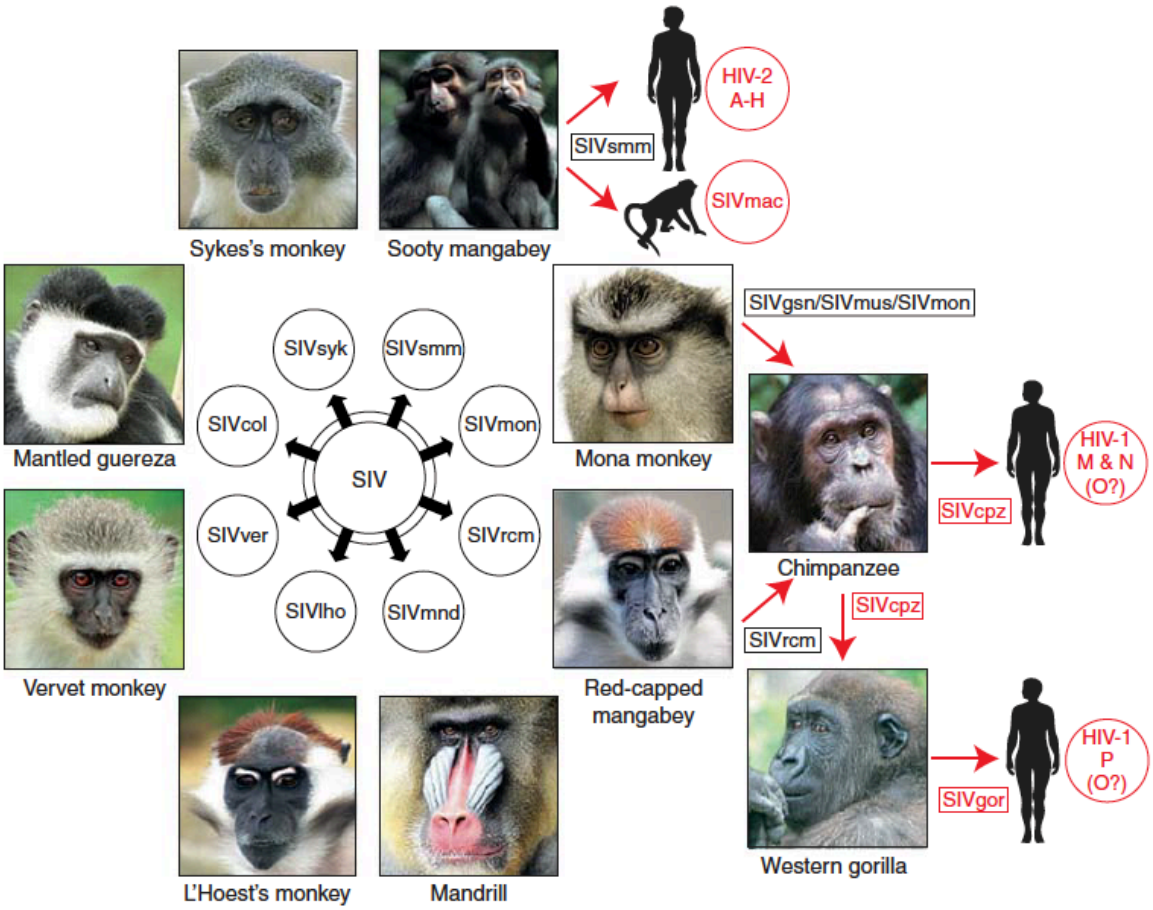
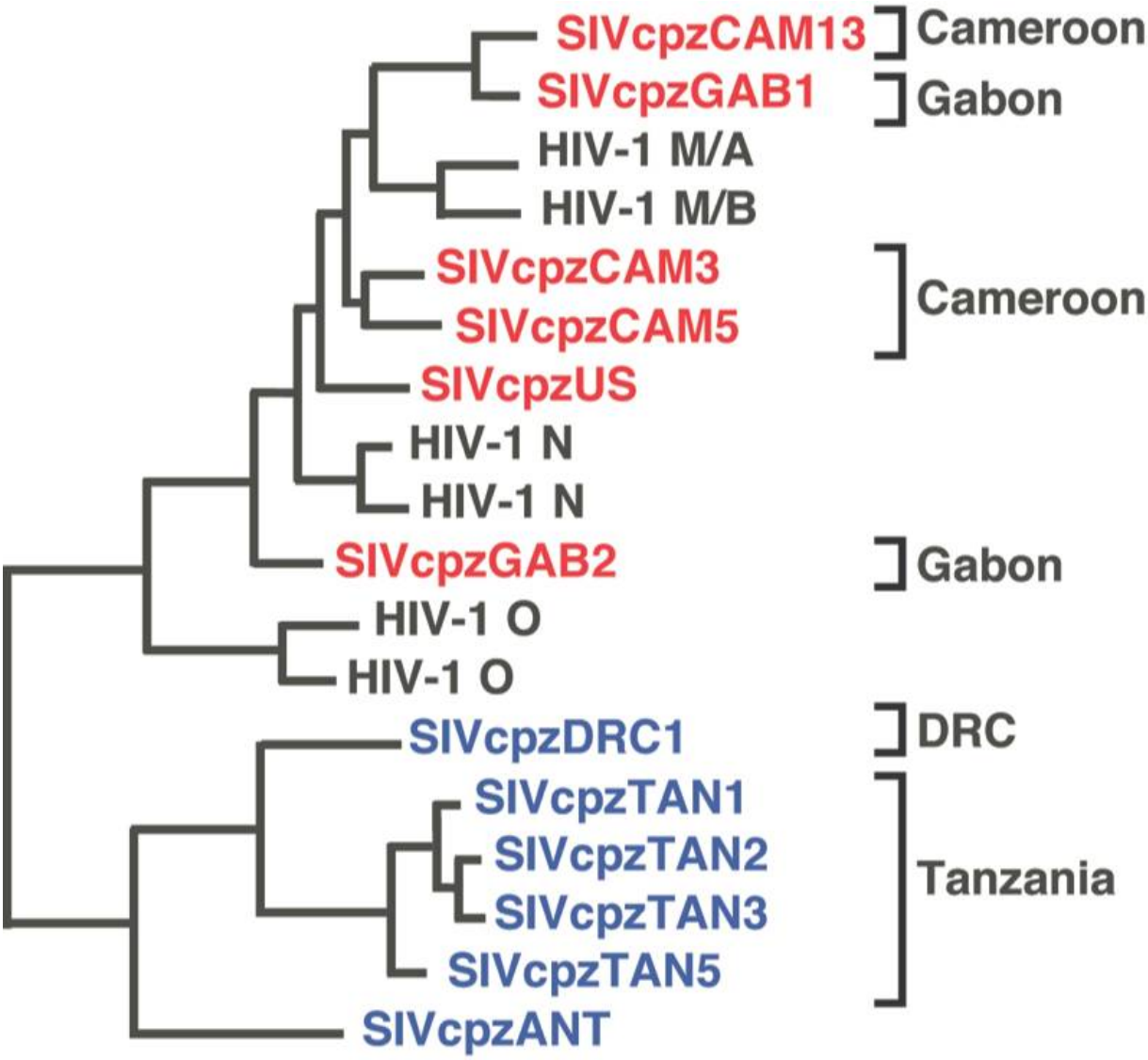


Figure 5. Phylogenetic and geographic relationships between SIVcpz (Simian Immunodeficiency Virus from Chimpanzees) and Human Immunodeficiency Virus (HIV) , adapted from [28] and [29] . The phylogeny is based on partial envelope region (180 amino acids). HIV-1 group M is nested between two SIVcpz clusters.



One of the main reasons for why the immune system cannot successfully control the HIV infection is the very fast mutation rate of HIV (10^{-4} to 10^{-5} per nucleotide)[30,31]. This high rate is explained by the lack of proofreading ability of the reverse-transcriptase (RT). It was estimated that every possible single-point mutation will occur at least once each day in an infected individual, when all the infectious particles are considered[32].

Main transmission routes and risk groups

Several transmission routes are known for HIV. Among them is sexual transmission, parenteral (either via infected needles or a blood transfusion) and a vertical transmission, *i.e.* from mother to child. The per sexual act transmission risk of HIV is not high, and was estimated to be around 2% (Table 1)[33]. This transmission risk is also not uniform when stratified by the type of sexual intercourse, with receptive anal sex having the highest estimated risk (138 per 10,000) and receptive oral the lowest (less than 4 infections per 10,000 exposures). The predominant transmission routes vary by geographic region. While in Europe and North America, the epidemic is driven by sexual transmission, that is mainly associated with Men-who-have-sex-with-Men (MSM), in Eastern Europe and Central Asia, 51% of all newly diagnosed HIV is attributed to people who inject drugs[1]. In Sub-Saharan Africa, which suffers from a generalized epidemic, with some countries, as South Africa, having a prevalence of 19.2% (18.4%-20.0%), the predominant transmission route is sexual among heterosexuals. Recently, there is a growing evidence that age-mixing patters, and specifically, sexual relationships between older men (aged 25–40) and young women (aged <25), are driving the epidemic in Africa[34,35].

Table 1. Risk of HIV infection by exposure route, adapted from [33].

Exposure route	Risk per 10 000 exposures to an infected source	95% Confidence interval
Parenteral exposure		
Blood transfusion	9250	(8900–9610)
Needle-sharing injection drug use	63[b]	(41–92)
Percutaneous needle stick	23	(0–46)
Sexual exposure[a]		
Receptive anal intercourse	138[c]	(102–186)
Insertive anal intercourse	11[d]	(4–28)
Receptive penile–vaginal intercourse	8[e]	(6–11)
Insertive penile–vaginal intercourse	4[e]	(1–14)
Receptive oral sex	Low[f]	(0–4)
Insertive oral sex	Low[f]	(0–4)
Vertical transmission		
Mother-to-child transmission	2260[g]	(1700–2900)

Highly-Active Antiretroviral therapy (HAART)

To date, except for the unique case of the "Berlin patient", HIV still cannot be cured [36]. This is because upon infection the virus integrates into the human immune cells, and can be re-activated from this latent reservoir even after several years. On the positive side, since 1996, a combination of antiretroviral drugs, can halt the diseases progression and allow individuals to reach life-expectancy that is almost identical to general population [37].

HAART can be effectively used not only to treat, but also to prevent the establishment of the infection, either as Post-Exposure Prophylaxis (PEP)[38]: administrating ART soon after potential exposure to the virus, or as Pre-Exposure-Prophylaxis (PreP)[39]: when a high-risk HIV negative individuals are prescribed antiretroviral drugs as protection from a possible exposure.

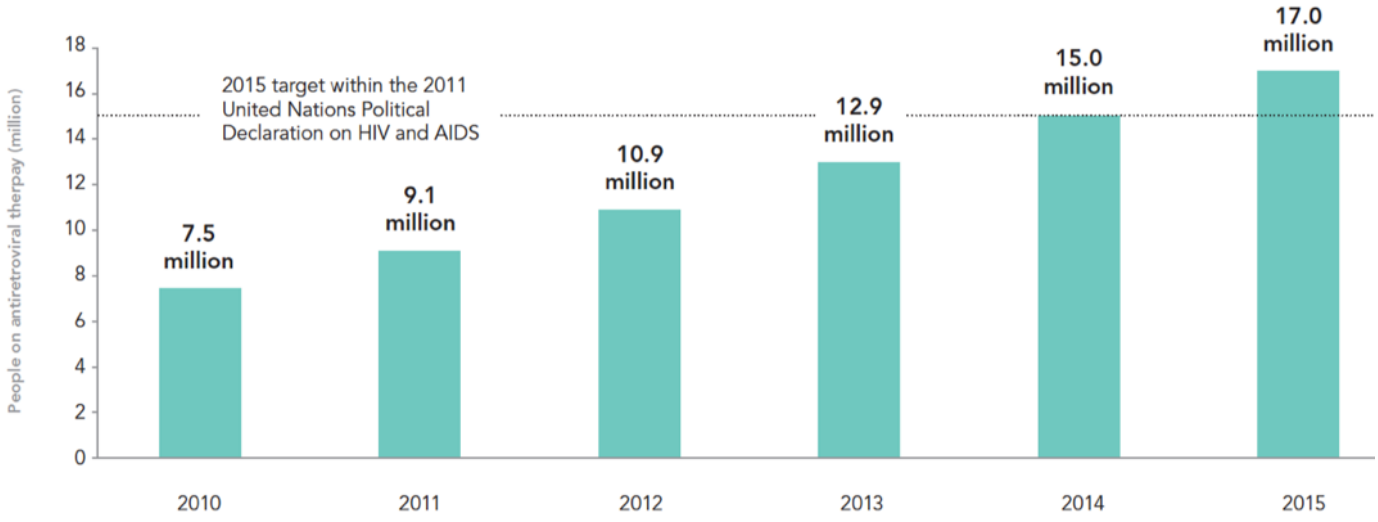
For treatment, the latest JAMA guidelines (2016), recommended HAART immediately upon HIV diagnosis regardless of CD4 cell counts[40]. This is primarily because of patient-level benefits in terms of a delayed time to AIDS[41] and reduced latent reservoir [42–44]. The beneficial effect of immediate ART was recently shown to include also the reduction in the risk of a broad spectrum of severe bacterial infections in HIV-positive people[45] with high CD4 cell count (above 500 cells per mL).

But there is also a population-level benefit, that stems from the concept of Treatment-as-Prevention (TasP), that is based on large clinical trials which demonstrated that virally suppressed HIV positive individuals are not infectious to others [41]. The recommended starting regimen is combining two nucleoside reverse transcriptase inhibitors (NRTIs) and an integrase strand transfer inhibitor (InSTI)[40].

Globally, in 2015, 36.7 million people were living with HIV, but only 60% of them were aware of their infection. As a result, only 17 million (46%) were actually on ART, with a very large variability between regions [46], and many are still not achieving viral suppression while on ART due to resistance, poor adherence or drug shortages. However, the trend of ART coverage is increasing (Figure 6).

In 2013, the UNAIDS set a target of "90-90-90", that by 2020, 90% of people living with HIV will know their status, 90% of the diagnosed will receive a sustained antiretroviral therapy, and 90% of people on ART will be virally suppressed[47].

Figure 6. The global number of people living with HIV on antiretroviral therapy, 2010-2015. Source [46].

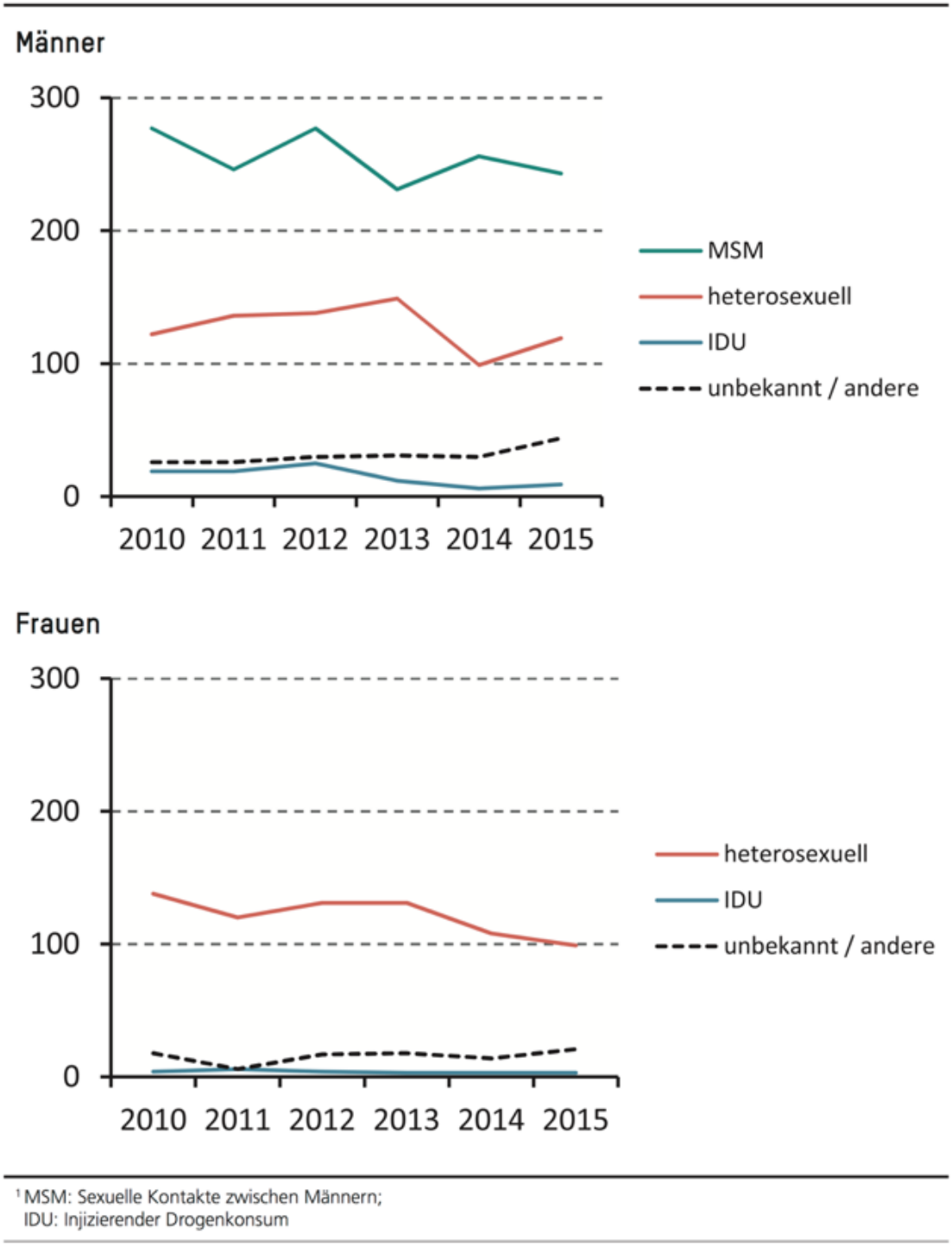


HIV in Switzerland

The HIV epidemic in Switzerland started early, with first AIDS related Kaposi sarcoma case reported in 1982, in Ticino, in a male patient that returned from the US [48]. Historically, the epidemic in Switzerland was driven by two main risk groups: Men-who-have-Sex-with-Men and injecting drug users. Kouyos et al. showed using phylogenetic analysis that there was probably no self-sustaining heterosexual epidemic in Switzerland[49]. Along the same line, Turk et al. showed a diminishing HIV transmission among Swiss heterosexuals far below the epidemic threshold[50]. As in other western countries the most prevalent HIV subtype in Switzerland is subtype B[26].

While the epidemic among IDU was contained, partly due to harm reduction, as described in chapter IV of this thesis, the HIV incidence among MSM remained relatively stable (Figure 7)[51].

Figure 7. Annual number of HIV diagnosis by sex and infection route, 2010 – 2015. Source: BAG [51]. Abbreviations: MSM – Men-who-have-Sex-with-Men, IDU – injecting drug-users.



The epidemiology of HIV in Switzerland is shaped by an interplay between behavioral and biological factors. On the social front, recent analysis showed an increase in condomless sex with occasional partners, among HIV positive MSM enrolled in the SHCS[52]. This trend is

potentially linked to the so called "Swiss Statement" that was made in 2008 which stated that the risk of HIV transmission for individuals on successful ART and that are in a stable partnership, is negligible[53]. While the direct causal effect of the statement on sexual risk behavior remains debated, there are several reports suggesting that the statement has contributed to increased condomless sex. For example, Shilaih *et al.* has showed that the incidence of syphilis in the Swiss HIV Cohort Study, strongly increased since 2008[54].

Antiretroviral Resistance in Switzerland

Unfortunately, due to the high mutation rate and a high within-patient diversity, the virus can develop and maintain resistance that can be either acquired or transmitted[55]. Acquired resistance is a result of poor adherence[56] or drug supply shortage[57]. Transmitted resistance, is found in patients that have never experienced HAART, but were infected with a resistant virus in the first place[58].

A recent analysis by Scherrer *et al.* that examined ART experienced patients enrolled in the Swiss HIV Cohort Study in the period 1999 to 2013, showed that emergence of acquired drug resistance almost stopped, with less than 0.4% of the patient that initiated ART after 2006, having a three-class resistance[59]. Concerning transmitted drug resistance (TDR) the median prevalence of resistance to any drug in the SHCS, from 1998 to 2012, was 9.1% (range, 2.2%-15.6%)[60]. The same study also demonstrated that transmission of antiretroviral drug resistance is transiently reduced by the introduction of new drug classes[60]. However, since the introduction of integrase-strand transfer inhibitor (INSTI), the resistance rates to this drug-class in Switzerland remained persistently low (0.1%)[61].

The interaction of HIV with other STIs

The epidemiological interplay between other sexually transmitted infections (STIs) and HIV can be bi-directional. On one hand, there is evidence suggesting that some STIs (*i.e* Syphilis, Gonorrhea, Genital Ulcer Disease (GOD)), can both increase the susceptibility of the HIV negative partner for the establishment of HIV infection and also increase the infectiousness of the HIV positive partner[62–64]. On the other hand, people that are already HIV positive, and especially Men-who-have-Sex-with-Men often remain in a high risk for the acquisition of additional STIs[54,65,66], due to continuous or cyclical sexual risk behavior[54]. In a cross-sectional study from Bern[65], Switzerland, the combined rates of laboratory-diagnosed and self-reported bacterial STI among HIV positive individuals in the year before the study were high: 27.7% (95% CI 21.1, 36.7%) among MSM, 1.5% (95% CI 0.2, 10.3%) among heterosexual men and 6.4% (95% CI 2.1, 21.0%) among women. The optimal STI screening frequency remains to be determined[67] which is further complicated by a high rate of asymptomatic STIs that can reach 90%[68].

Phylogenetic studies of HIV

A phylogenetic tree depicts the evolutionary history and relatedness between species, between individuals, or even between individual genes. In a perfectly sampled population, the closer are two members on the tree, the more closely they are related to the same common ancestor. In the HIV field, phylogenetic approaches were successfully applied to a plethora of research questions. Among the most frequent applications are:

(i) Shading light on evolutionary history and introduction events. For example, it was shown that 1920s Kinshasa (in what is now the Democratic Republic of Congo) was the source of the early HIV-1 spread in human populations[69]. Another phylogenetic work showed that HIV-1 subtype B USA epidemic, from where HIV was later introduced to Europe[70], originated from a

pre-existing Caribbean epidemic[71]. This jump to the USA was estimated to have happened via New York, between 1969–1974[71].

(ii) Utilizing phylogeny for elucidation of transmission dynamics and epidemiology. This research front swiftly emerged in the last decade and is fueled by a dramatic increase in the availability of HIV sequences, as a byproduct of a clinical drug resistance testing to optimize treatment[72]. In Switzerland alone, phylogeny was already used to elucidate the degree of mixing between different risk groups[49], to identify drivers of onward transmission[73] and to calculate the basic reproductive number (R_0) of HIV[74], which was estimated to be 2.29 [75]. For the heterosexual population, the R_0 was recently estimated to be 0.44 (95%-confidence interval 0.42-0.46), which is far below the epidemic threshold[50].

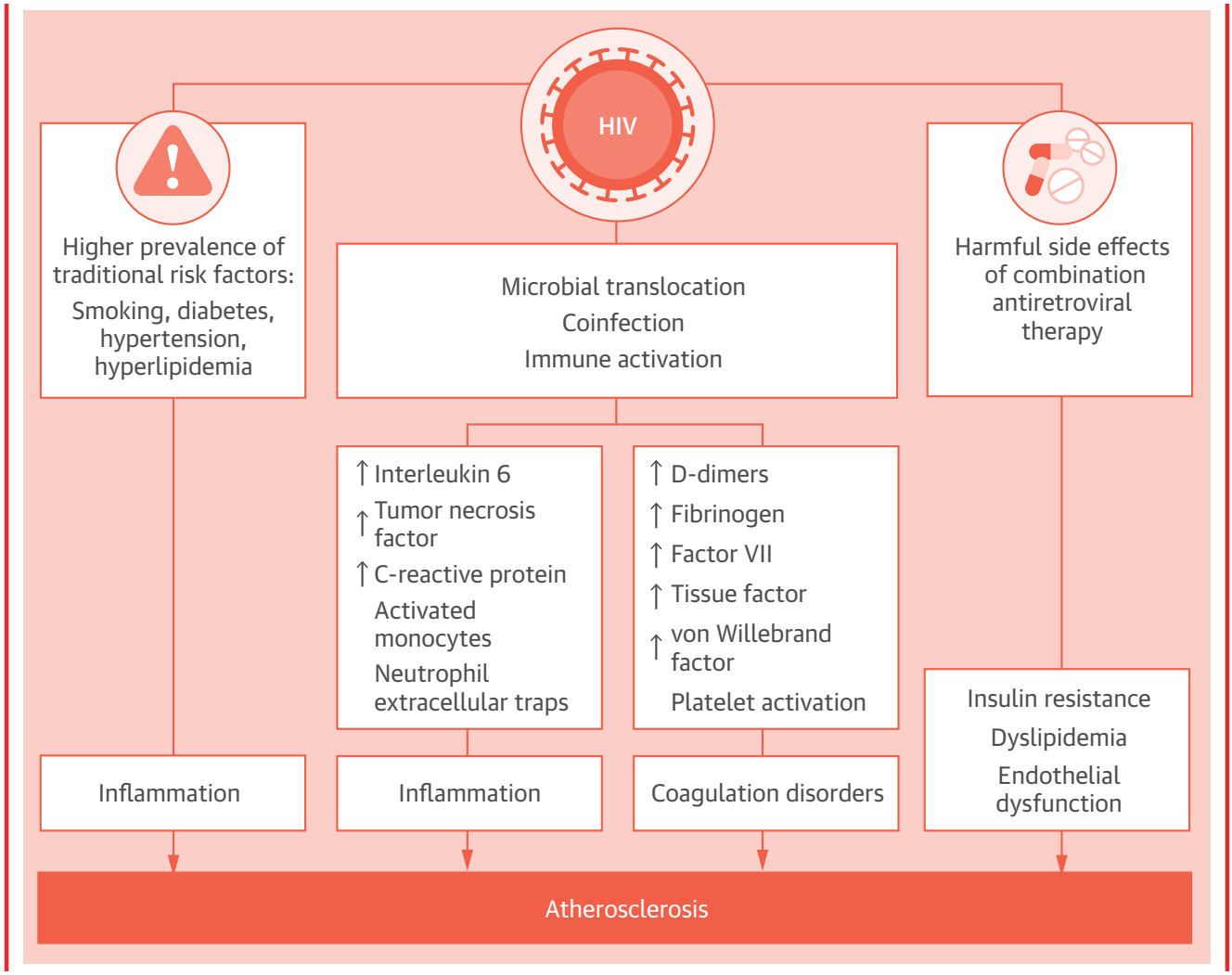
Since incomplete sampling can be rarely ruled out, phylogenetic proximity cannot be interpreted as a direct transmission event[76,77], which is a widely recognized limitation.

(iii) Detecting heritability of disease related traits. In other words, what fraction of a trait variance can be explained by viral genetics as opposed to host related factors. By implementing phylogenetic methods, it was shown that viral genetics partly determines the set point viral load[13], the rate of decline of the CD4 cells[14] and the virus load adjusted pathogenicity[17].

HIV as chronic disease: Aging, Ischemic Heart disease and Nutrition

Although life expectancy in HIV-positive people on ART has improved worldwide in recent years, important gaps still remain in comparison with the general and HIV-negative population[78]. Studies have shown that in comparison to HIV uninfected individuals, HIV positive people suffer from higher incidence of cardiovascular disease (CVD), bone, metabolic, neurocognitive and other aging comorbidities[79,80,81]. HIV-associated inflammation, persistent immunodeficiency, cumulative toxic effects from exposure to antiretroviral drugs, all have been implicated as potential causes[82]. In the D:A:D: Study it was shown that the incidence of myocardial infarction increased with cumulative exposure to ART[83]. In a recent study, subclinical coronary artery stenosis was also associated with a longer treatment with HAART[84]. HAART is associated with HIV lipodystrophy, dyslipidemia, diabetes mellitus and insulin resistance[85]. However, those effects strongly depend on the individual drug and side-effects are more benign for newer regiments[86]. Nevertheless, all these metabolic disturbances contribute to the elevated risk of CVD[87]. This adds up to the fact that HIV positive individuals generally tend to have a high rate of traditional risk factors for CVD like smoking and hyperlipidemia[88], Figure 8. Increased obesity rates were recently demonstrated in the Swiss HIV Cohort Study as well[89]. All this makes understanding the contribution of dietary habits and physical activity a pertinent task which carries a possible prevention potential[90,91], as those are modifiable risk factors.

Figure 8. HIV and Ischemic Heart Diseases: Etiopathogenesis of HIV-Associated Coronary Artery Diseases, adapted from[88]:



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CHAPTER I

"HIV-1 Transmission During Recent Infection and During Treatment Interruptions as Major Drivers of New Infections in the Swiss HIV Cohort Study"

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Detailed Personal Contribution

The project was conceptualized by RDK, HFG and AM. AM extracted the data from the SHCS patient and resistance databases, performed quality control, constructed the phylogenetic trees, and analyzed the data using various statistical tools, plots, and methods. AM produced all the tables and figures. AM wrote the first manuscript draft and the final version.

Research in context

The impact of transmission during recent HIV infection is a crucial determinant of the success of Treatment-as-Prevention (i.e. treating HIV early not only for the benefit of the individual but also to prevent onward transmission, since treated individuals are not infectious) as a strategy to curb the HIV pandemic, because recently infected patients are often unaware of their infection and therefore remain untreated. Yet, previous studies have shown discrepant estimations of the fraction of transmissions attributable to recent infection ranging from less than 10% to 70–80%. In this work we combined the unique data from the Swiss HIV Cohort Study (SHCS), the associated drug resistance database and the Zurich Primary HIV Infection Study (ZPHI) to empirically determine the impact of recent-phase transmission in a representative population: Using molecular epidemiology approaches, we identified transmission-pairs with available seroconversion dates and found that over 40% of transmitters transmitted the virus in the first year of infection and over 30% within the first 6 months of infection. Transmission during the chronic infection phase was strongly and significantly associated with late-start of antiretroviral therapy (ART) and high chronic phase virus load, underlining the importance of ART for preventing chronic-phase transmission. Finally, a substantial fraction (>15%) of chronic phase transmission events occurred during treatment interruptions. These findings underline that Treatment-as-Prevention should be complemented by efforts for early diagnosis and measures for optimizing treatment continuity. Interestingly, four months after this study was published, a research group from the Imperial College London published[1] an independent analysis of the ATHENA cohort from the Netherlands, in which they found that 43% of all transmitters transmit within the first year of infection, which matches exactly our reported estimate.

Reference

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Clinical Infectious Diseases

MAJOR ARTICLE

HIV/AIDS

HIV-1 Transmission During Recent Infection and During Treatment Interruptions as Major Drivers of New Infections in the Swiss HIV Cohort Study

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Background. Reducing the fraction of transmissions during recent human immunodeficiency virus (HIV) infection is essential for the population-level success of "treatment as prevention".

Methods. A phylogenetic tree was constructed with 19 604 Swiss sequences and 90 994 non-Swiss background sequences. Swiss transmission pairs were identified using 104 combinations of genetic distance (1%–2.5%) and bootstrap (50%–100%) thresholds, to examine the effect of those criteria. Monophyletic pairs were classified as recent or chronic transmission based on the time interval between estimated seroconversion dates. Logistic regression with adjustment for clinical and demographic characteristics was used to identify risk factors associated with transmission during recent or chronic infection.

Findings. Seroconversion dates were estimated for 4079 patients on the phylogeny, and comprised between 71 (distance, 1%; bootstrap, 100%) to 378 transmission pairs (distance, 2.5%; bootstrap, 50%). We found that 43.7% (range, 41%–56%) of the transmissions occurred during the first year of infection. Stricter phylogenetic definition of transmission pairs was associated with higher recent-phase transmission fraction. Chronic-phase viral load area under the curve (adjusted odds ratio, 3; 95% confidence interval, 1.64–5.48) and time to antiretroviral therapy (ART) start (adjusted odds ratio 1.4/y; 1.11–1.77) were associated with chronic-phase transmission as opposed to recent transmission. Importantly, at least 14% of the chronic-phase transmission events occurred after the transmitter had interrupted ART.

Conclusions. We demonstrate a high fraction of transmission during recent HIV infection but also chronic transmissions after interruption of ART in Switzerland. Both represent key issues for treatment as prevention and underline the importance of early diagnosis and of early and continuous treatment.

Keywords. HIV recent (early) infection; treatment as prevention; treatment interruptions; HIV transmission; endgame.

Human immunodeficiency virus (HIV) remains an immense public health threat, with a global prevalence of 35.3 million infected individuals in 2013 [1]. Whereas in most high-income countries the incidence of male-female transmission has been stable or decreasing, the incidence of male-male transmission is rising or remains high [2]. In this context, one pivotal question is the relative contribution of the early and chronic disease phases to HIV transmission. Previous studies have shown discrepant estimations of the fraction of transmissions attributable to recent infection ranging from <10% [3] to 70%–80% [4].

Knowing the burden of early-phase transmission is important for public health policy, especially in the context of the latest efforts to introduce immediate and early antiretroviral therapy (ART)—that is, "treatment as prevention" (TasP)—as one of the main global containment strategies of the HIV pandemic [5]. A growing body of evidence suggests that once an HIV-positive individual is diagnosed and successfully treated with ART, the hazard of onward transmission drops dramatically [6,7].

A high proportion of recent-phase HIV transmissions will compromise the effectiveness of TasP for several reasons. First, a substantial fraction of recently infected patients are still unaware of their HIV-positive status and thus remain untreated and infectious [8]. Secondly, infectiousness during primary HIV-infection has been estimated to be up to 26 times higher than during later (pre-AIDS) stages of the infection [9], which is further supported by a higher HIV-1 concentration in semen [10]. Finally, ongoing transmission of drug-resistant viral variants by patients unaware of their infection may compromise the effectiveness of ART [11].

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In this work, we addressed this question by retrospectively analyzing transmission pairs from the unique data from the Swiss HIV Cohort Study (SHCS), the associated drug resistance database, and the Zurich Primary HIV Infection Study (ZPHI). The aims of this study were to determine the fraction of HIV transmissions that occurs during recent infection and to evaluate HIV transmission in relation to the timing of ART initiation.

METHODS

Study Population: SHCS Drug Resistance Database and ZPHI

The SHCS is a large prospective multicentered, study established in 1988 [12]. During the biannual outpatient follow-up visits, extensive clinical and demographic data are collected for each participant. The drug resistance database contains HIV sequences for approximately 60% of the patients in the SHCS. The SHCS is highly representative of the HIV epidemic in Switzerland, with an estimated coverage of ≥45% of all HIV cases, 69% of all patients with AIDS in Switzerland and 72% of all ART-treated individuals [12]. The ZPHI [13, 14], specifically enrolls patients with documented acute or recent primary HIV-1 infection.

Phylogenetic Tree Construction

A total of 19 604 partial *pol* sequences from 10 970 SHCS cohort participants (40% of patients had ≥2 sequences) were pooled with 90 994 background sequences from the Los Alamos database. The phylogenetic tree was generated (see Supplementary Text 2 for details) with FastTree software (version 2.1.7, SSE3, OpenMP) [15] using a generalized time-reversible model. Support values for internal nodes were derived based on 100 bootstrapped trees. With use of the R package APE (version 3.1) [16] and custom scripts, potential transmission pairs were identified as monophyletic pairs if their genetic distance and bootstrap support values met the predefined thresholds of 104 combinations of genetic distance (1%, 1.5%, 2%, and 2.5%) and bootstrap (50%–100% in 2% increments) support. This was done to estimate the effect of various transmission cluster definitions on the dependent variable, because there is no consensus on optimal thresholds [17].

Estimation of Infection Dates

Seroconversion dates were estimated based on a hierarchical algorithm (Figure 1; see Supplementary Text 1 for detailed description), which relied on participation in the ZPHI,

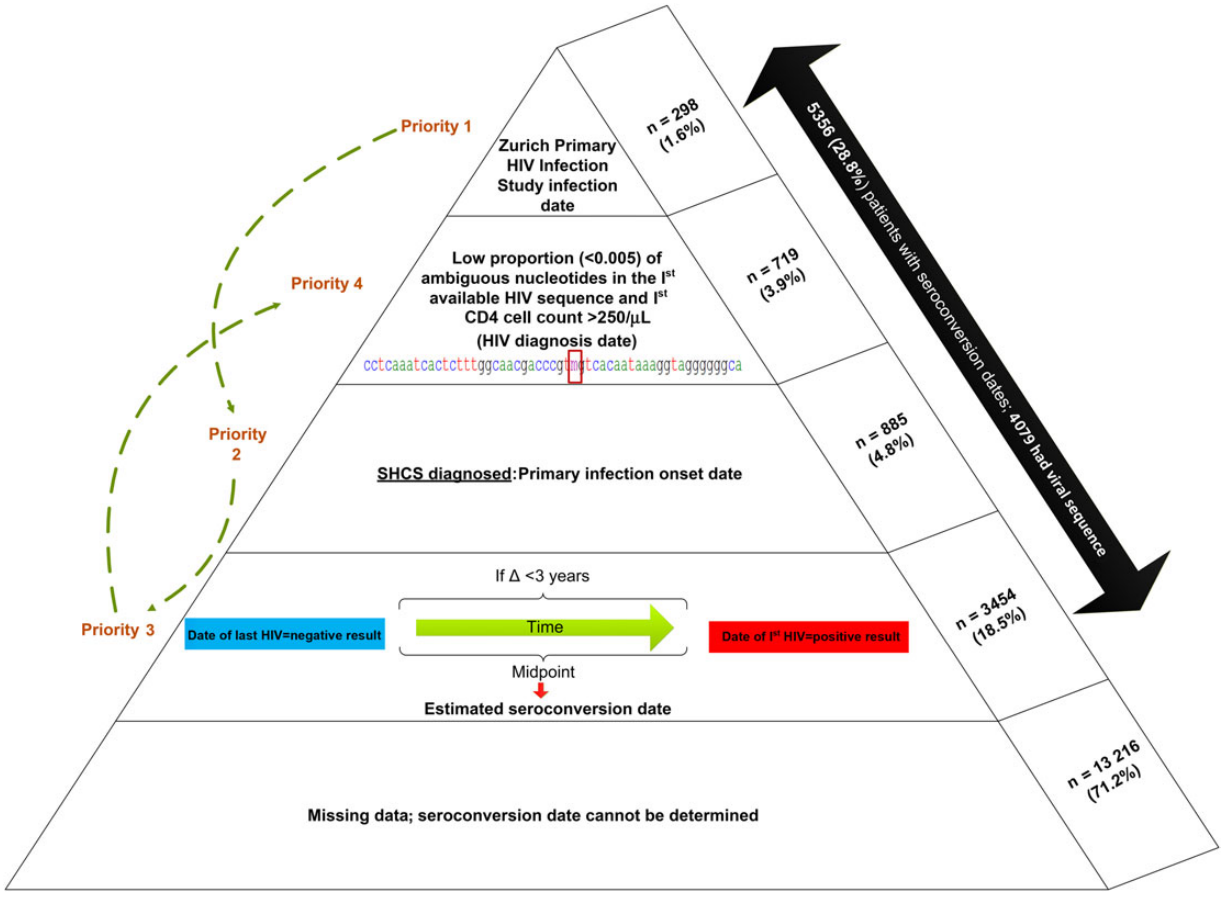

Figure 1. Hierarchical algorithm for the determination of the infection dates for patients enrolled in the Swiss HIV Cohort Study (SHCS) (n = 18 572). Abbreviation: HIV, human immunodeficiency virus.

Table 1. Clinical and Demographic Characteristics of the Study Population

Characteristic	All SHCS	Phylogenetic Tree	Distance: 1%; Bootstrap: 100%[a]	Distance 1.5%; Bootstrap: 80%[a]	Distance 2.5%; Bootstrap: 50%[a]
Patients, No.	18 572	10 970	142	428	744
Age at diagnosis, mean (IQR), y	34 (26.4–40)	34.5 (26.5–40)	36.4 (29–42)	36 (28.6–42)	35.3 (28–41)
Sex, No. (%)					
Male	13 369 (71.98)	7799 (71.09)	122 (85.92)	363 (84.81)	632 (84.95)
Female	5203 (28.02)	3171 (28.91)	20 (14.08)	65 (15.19)	112 (15.05)
Risk group, No. (%)					
MSM	6929 (37.3)	4205 (38.3)	93 (65.5)	271 (63.3)	463 (62.2)
Heterosexuals	6118 (32.9)	3919 (35.7)	40 (28.2)	102 (23.8)	158 (21.2)
Injection drug users	3281 (17.7)	1627 (14.8)	6 (4.2)	35 (8.2)	76 (10.2)
Other	2244 (12.1)	1219 (11.1)	3 (2.1)	20 (4.7)	47 (6.3)
Subtype, No. (%)					
B	8314 (75.8)	8314 (75.8)	96 (67.6)	335 (78.3)	616 (82.8)
Non-B	2656 (24.2)	2656 (24.2)	46 (32.4)	93 (21.7)	128 (17.2)
Ethnicity					
White	12 528 (67.5)	8495 (77.5)	116 (81.7)	371 (86.9)	660 (88.9)
Other	6039 (32.5)	2472 (22.5)	26 (18.3)	56 (13.1)	82 (11.1)
RNA viral load, median (IQR),[b] log_{10} copies/mL	4.65 (3.96–5.2)	4.65 (4–5.2)	4.81 (4–5.41)	4.82 (4.18–5.45)	4.76 (4.11–5.36)
CD4 cell counts, median (IQR), cells/μL [b]	342 (167–546)	370 (200–562)	420 (291–622)	440 (302–636.5)	471 (319.5–655.5)
ART start year, median (range)	1999 (1986–2014)	2000 (1986–2014)	2009 (1990–2013)	2008 (1990–2013)	2008 (1990–2014)
Time to ART, median (IQR), mo	20.8 (6.03–50.7)	21.5 (6–51.55)	14.5 (2.68–32.93)	14.7 (3.1–36.6)	16.6 (4.27–40.58)
Cohort recruitment year, median (range)	1997 (1981–2014)	2000 (1984–2014)	2008 (1989–2013)	2007 (1989–2013)	2006 (1987–2013)
Progressed to AIDS, No. (%)	6657 (35.8)	3032 (27.6)	11 (7.7)	40 (9.3)	73 (9.8)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; SHCS, Swiss HIV Cohort Study.

[a] Based on pairs with available seroconversion dates.

[b] Earliest treatment-naive measurement after enrollment.

immunological markers, dates of HIV-positive/negative tests, clinical symptoms and ambiguous nucleotides [18, 19].

Classification of Transmission Pairs as Recent or Chronic

After construction of the phylogenetic tree and the estimations of the patients' infection dates, a time interval between the infection dates of the members of each phylogenetically established transmission pair was calculated (Supplementary Figure 1). Clusters with an interval of ≤6 or ≤12 months within a pair were classified as recent transmission (to account for variable definitions of the duration of recent infection [20], 2 analyses were performed, 1 for each threshold), and those with an interval of >6 or >12 months as chronic transmission.

Determining the Potential Transmitter and the Infection Window of the Recipient Within Each Pair

By default, a "transmitter" was defined as the member of the pair with the earliest seroconversion date. For the analysis of transmission in relation to time of ART initiation, we also defined for each potential recipient the most plausible infection window. Its upper bound is given by the first positive HIV test. Its lower bound is given by the latest of 3 dates: (1) 90 days before the last HIV negative test; (2) for individuals with a diagnosis of primary HIV infection (categories I and II in Supplementary Text 1), 365 days before the first HIV positive test; and (3) 730 days before the first positive test, if the patients' seroconversion date was estimated based on a resistance test with <0.5% of ambiguous nucleotides within 3 years after diagnosis and a first CD4 cell count >250/μL [18].

Estimation of Infectiousness During the Chronic Phase

Among the phylogenetically inferred transmitters, we identified risk factors associated with the relative odds of being a chronic- or recent-phase transmitter using logistic regression. To quantify the transmission potential [21] during the chronic phase, which depends both on viral load (VL) magnitude and the duration with detectable VL, we calculated for each patient with ≥2 chronic-phase RNA measurements, the area under the curve (AUC) of the log_{10}-transformed RNA VL from the end of the recent infection (1 year after the seroconversion date) to the last laboratory result of the chronic phase. To facilitate the regression interpretation, this variable was standardized (such that its mean is zero and one unit is one standard deviation). For the comparison of chronic-phase post-ART and pre-ART transmitters, VL AUC was calculated from the time of ART initiation to the last RNA measurement.

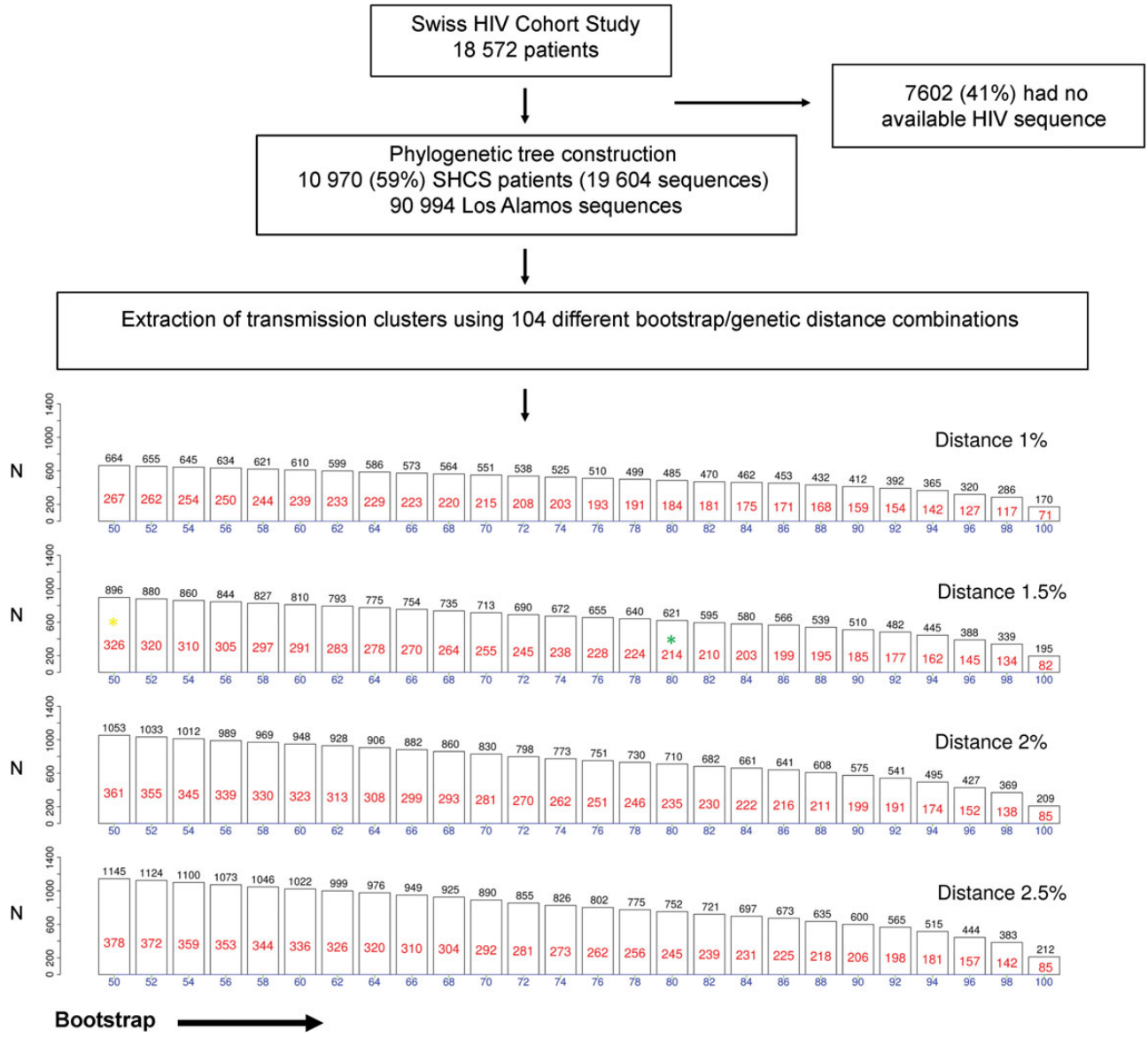

Figure 2. Outline of this study. Each bar represents a different combination of bootstrap and genetic distance thresholds. Black numbers above the bars represent the number of transmission pairs that correspond to the specific combination; red numbers, the number with available seroconversion dates for both members; blue numbers at the x-axis, ascending bootstrap thresholds; green and yellow asterisks, data sets used for the main logistic regression and sensitivity analyses, respectively. Abbreviations: HIV, human immunodeficiency virus; N, number of pairs; SHCS, Swiss HIV Cohort Study.

Statistical Analysis

Statistical analysis was performed with R software (version 3.0.3; http://cran.r-project.org).

Ethics

Ethical approval of the SHCS and the ZPHI and written informed consent for all participants were obtained.

RESULTS

Data Description

Of 18 572 SHCS participants, 10 970 (59%) had ≥1 sequence in the SHCS drug resistance database and were hence considered for further analysis (Table 1). Their year of HIV diagnosis ranged from 1984 to 2014. Of these patients, 7799 (71%), were men, 8314 (75%) were infected with subtype B, 4205 (38%) were men who have sex with men (MSM), and 8495 (77%) were white. Depending on the phylogenetic threshold, 3%–20.6% of the patients represented on the phylogenetic tree were members of a putative transmission pair (Figure 2). Seroconversion dates could be estimated for 4079 patients represented on the phylogeny, 82% with diagnosis during the first year after seroconversion. As expected, stricter bootstrap thresholds were associated with fewer transmission pairs (Figure 2) (Spearman $\rho = -1$; $P < .001$ for all 4 distances). For all 104 phylogenetic thresholds the predominant risk group among transmission pairs was MSM, ranging between 62% and 66%.

Estimation of HIV Transmission During Recent Infection

To estimate the fraction of transmissions attributable to recent infections, we selected potential transmission pairs using 104 different combinations of bootstrap and genetic distance. For each combination, we calculated the fraction of the recent-phase transmission (see "Methods" section). Overall, we found a high fraction of transmission during recent infection. This fraction was higher, but not proportionally higher, when recent infection was defined as first year of infection (vs the first 6 months) and increased with the strictness of the criterion used to define transmission pairs. When recent HIV infection was defined as the first year since seroconversion, the median fraction of transmission during recent infection was 43.7% and ranged from a minimum of 41% (95% confidence interval [CI], 36%–46%) for a bootstrap of 50% and a distance of 2.5% to a maximum of 56.5% (95% CI, 45%–67%) for a bootstrap of 100% and distances of 2% and 2.5%. When recent HIV infection was defined as 6 months since seroconversion, the median fraction of transmission during recent infection was lower (31.6%) and ranged from a minimum of 28% (95% CI, 23%–33%) with a bootstrap of 50% and distance of 2.5% to a maximum of 42.3% (95% CI, 32%–54%) with a bootstrap of 100% and distances of 2% and 2.5%.

For all 4 distance thresholds, a positive correlation was observed between the bootstrap thresholds and the recent-phase (12-month) transmission fractions (Figure 3) (Spearman $\rho > 0.70$; $P < .001$). Thus, a higher bootstrap threshold resulted in a higher fraction of recent-phase transmission. Importantly, the fraction of recent transmission increased sharply for higher bootstrap values (>92%), indicating that high bootstrap thresholds may bias the selection toward recently infected transmission pairs. For the 6-month definition of recent HIV infection the correlation between bootstrap and the fraction of attributable recent-phase transmissions was even stronger, and was significant for all 4 genetic distances tested (Spearman $\rho > 0.93$; $P < .001$). Thus, our phylogenetic analysis indicates that a large share of infections can be attributed to recent-phase transmission but that the exact proportion varies depending on the definition of a transmission pair (bootstrap and distance thresholds) and the duration of recent infection (12 vs 6 months).

Risk Factors for Chronic Transmission

HIV-1 transmission in the chronic phase, as opposed to the recent phase, was strongly associated with higher AUC of chronic-phase VL and delayed initiation of ART. Logistic regression was applied to the data set that corresponded to a genetic distance of 1.5% and bootstrap of 80% (Table 2). These thresholds were chosen as a compromise between 3 criteria: (1) avoiding the above-mentioned selection bias toward recent infection,

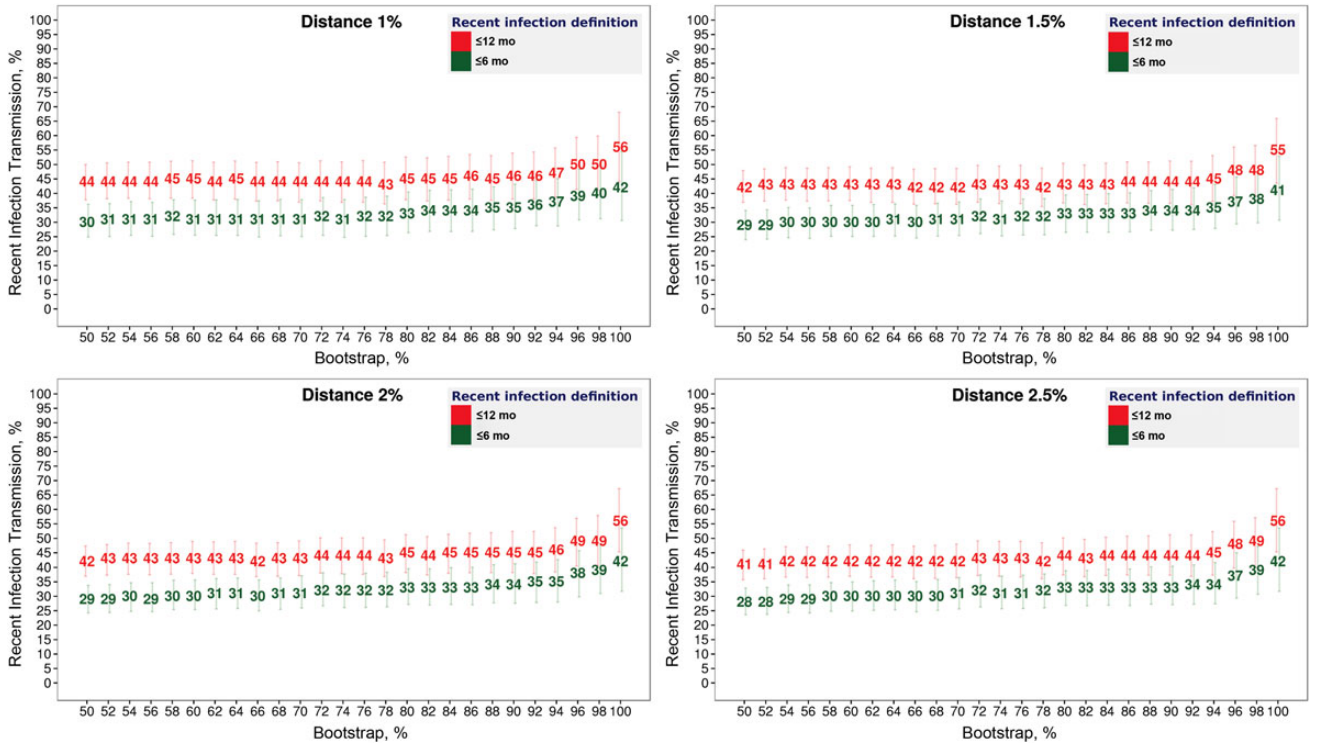

Figure 3. Swiss HIV Cohort Study–based estimation of transmission during recent human immunodeficiency virus infection. Red numbers represent the fraction of transmissions during recent infection, according to a definition of recent infection as 12 months since seroconversion; green numbers, recent transmission fraction for a definition of 6 months since seroconversion. In all, 104 combinations of genetic distance (1%, 1.5%, 2%, or 2.5%) and bootstrap (50%–100% in 2% increments) support values are shown; vertical lines represent 95% confidence intervals for proportion. Abbreviation: HIV, human immunodeficiency virus.

Table 2. Logistic Regression Analysis for Chronic Versus Recent Phylogenetically Linked Human Immunodeficiency Virus Transmitters[a]

Variable	Bivariate OR (95% CI)	*P* Value	Multivariable OR (95% CI)	*P* Value
Age at infection	0.96 (.93–.99)	.01	0.97 (.93–1.01)	.11
Sex				
Male	Reference	. . .	. . .	. . .
Female	1.04 (.49–2.23)	.92	2.43 (.6–9.94)	.22
Risk group				
MSM	Reference	. . .	. . .	. . .
Heterosexuals	1.08 (.56–2.1)	.81	0.61 (.15–2.52)	.49
Injection drug users	0.92 (.33–2.51)	.86	0.54 (.09–3.38)	.51
Subtype				
Non-B	Reference	. . .	. . .	. . .
B	1.4 (.72–2.73)	.32	1.28 (.45–3.67)	.65
$\sqrt{}$CD4 cell counts[b]	1.02 (.98–1.07)	.34	0.93 (.87–1)	.04
Transmission year	0.99 (.93–1.06)	.82	1.13 (1.02–1.26)	.02
Time to ART (years)	1.5 (1.26–1.8)	<.001	1.4 (1.11–1.77)	.005
Chronic RNA VL AUC	2.62 (1.74–3.97)	<.001	3 (1.64–5.48)	<.001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; MSM, men who have sex with men; OR, odds ratio; VL AUC, viral load area under the curve.

[a] Of 170 transmitters (complete cases), 94 were chronic and 76 recent. Chronic transmission was defined as >1 year since seroconversion (coded as 1); recent transmission, as ≤1 year (coded as 0); phylogenetic linkage thresholds: bootstrap, 80%, genetic distance 1.5%.

[b] Earliest treatment-naive measurement after enrollment.

which occurs for very strict criteria; (2) providing a fair statistical power (170 complete cases); and (3) minimizing the probability of false-positive clustering. In a bivariate analysis, transmitting HIV during chronic infection was positively correlated with time until the initiation of ART (odds ratio, 1.5/y; 95% CI, 1.26–1.8) and with higher chronic-phase VL AUC (2.62; 95% CI, 1.74–3.97) (Table 2). In the multivariable model, both time to ART and the AUC of HIV plasma RNA during the chronic phase remained significantly associated with higher odds of chronic as opposed to recent transmission (adjusted odds ratio, 1.4/y [95% CI, 1.11–1.77] and 3 [1.64–5.48], respectively). Thus 1 standard-unit change in chronic VL AUC was associated with 3-fold increased odds of chronic-phase HIV transmission compared with recent-phase transmission, after adjustment for potential confounders and time to initiation of ART. Moreover, we found—only in the multivariable analysis—that later transmission years were associated with chronic-phase transmission, and higher baseline CD4 cell counts with recent transmission. In a sensitivity analysis, we found similar results with the more lenient criteria of 1.5% distance and 50% bootstrap (Supplementary Table 1). In summary, we showed that increased delay to initiation of ART shifts the relative odds of transmission toward the chronic phase. Moreover, our data indicate that the total RNA VL in the chronic phase increases the relative odds of transmitting HIV during this phase, even after adjustment for treatment initiation.

Transmission in Relation to ART Initiation

To explain the above-mentioned, ART-adjusted association of total chronic-phase VL with chronic-phase transmission, we further examined the chronic-phase transmitters (n = 121) in relation to ART initiation. Our data show that a substantial fraction of chronic-phase transmission occurred after ART was started by the transmitter.

For 54 of 121 chronic-phase transmitters (45%), the seroconversion date of the recipient was after the ART initiation date of the transmitter. In line with post-ART transmission, the mean post-ART VL AUC of post-ART transmitters was higher than that of pre-ART transmitters (0.17 vs −0.38; $P = .002$, Wilcoxon rank sum test) (Figure 4). Restricting the transmitters' VL measurements only to those obtained during the recipients' infection window (see "Methods" section) further corroborated transmission after ART: 44 of 54 transmitters had ≥1 VL measurement in the relevant period, and 35 of these 44 transmitters had ≥1 VL value >400 copies/mL [22] with a median nonzero maximal VL of 70 800 copies/mL (range, 2340 to 4.99×10^6 copies/mL). The

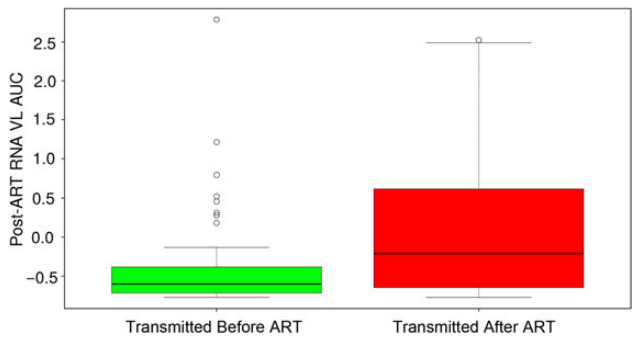

Figure 4. Total post–antiretroviral therapy (ART) viral load area under the curve (VL AUC) of pre-ART (*green*) and post-ART (*red*) transmitters. The median post-ART VL AUC of post-ART transmitters was higher than that of pre-ART transmitters (based on 121 chronic transmitters selected using a bootstrap of 80% and a genetic distance of 1.5%).

remaining 9 transmitters might represent a nondirect transmission pair (eg, with a missing intermediate transmitter) or a false-positive cluster; alternatively, the intermittent VL rebounds might have been missed by the 3–4 monthly VL measurements.

Finally, we determined in more detail the treatment status of the 35 VL-confirmed post-ART transmitters. For 18 transmitters, the date of ART initiation for the transmitter lay within the transmission window for the recipient. Hence, even though the estimated seroconversion date suggests post-ART transmission, we cannot exclude for those patients the possibility that the transmission occurred shortly before ART (Supplementary Figure 2*A*). Thus, these individuals transmitted either briefly before or briefly after ART initiation. For the remaining 17 transmitters, the transmitter's date of ART initiation lay completely before the recipients' infection window (Supplementary Figure 2*B*). Importantly, 16 of 17 had a documented period of treatment interruption during the recipient's infection window. These therapy interruptions lasted between 42 to 859 days within the infection window of the recipient. The remaining transmitter had no documented treatment interruption but carried high-level resistance mutations (M184V and K103N) and did not achieve viral suppression in the 6 years after treatment initiation, including the recipient's transmission window.

Overall, these results indicate that a substantial fraction of chronic-phase transmission events—at least 17 of 121 (14%) and up to 54 of 121 (45%)—occurred after ART initiation by the transmitter. This observation underlines the important contribution of treatment interruptions and the periods close to ART initiation for onward HIV transmission.

DISCUSSION

In Switzerland, despite increasing treatment coverage and decreased time to ART initiation, the number of newly diagnosed HIV infections remains stable [23]. Our study revealed 2 key challenges for achieving a population level effect of TasP: recent infections and HIV transmission during treatment interruptions in patients with chronic infection.

We demonstrated that a substantial fraction of HIV transmissions in the SHCS can be attributed to recently infected patients, for whom the preventive effect of treatment is weaker, due to underdiagnosis and lack of patient's awareness of his seropositive status. In addition, immediate treatment of acute or recent infection was recommended only recently [24]. Moreover, our data show a strong effect of total VL on transmission in the chronic phase, even after adjustment for time to initiation of ART. This effect is partly due to transmission after ART initiation, notably during treatment interruptions. This observation implies that rapid administration of treatment, while the patient is still in the early phase of infection, is necessary but not sufficient to prevent transmission (because transmission may also occur after ART interruption in the chronic phase).

Our findings imply that TasP needs to be accompanied by interventions to tackle treatment continuity, adherence, retention in care, and, importantly, early diagnosis [25, 26]. A systematic review has shown that the median proportion of patients interrupting treatment was 23% for a median duration of 150 days [27]. Furthermore, 54% of HIV-diagnosed patients in Europe were late presenters—individuals who had a CD4 cell count <350/μL or an AIDS-defining illness within 6 months of HIV diagnosis [28]. Cumulatively, our data imply that treatment interruptions, whether structured or due to toxic effects, patient's wishes, or lack of adherence, are not only unfavorable for the individual [29] but also bear public health consequences [26].

Our work further underlines the need for validated and consensual thresholds for phylogeny-based detection of HIV transmission. The observed positive correlation between the strictness of the transmission pair selection criteria (higher bootstrap and lower genetic distance) and the fraction of transmissions attributed to recent infections implies that too-strict selection criteria overestimate the fraction of recent-phase transmission. Several other studies that implemented strict genetic distance and bootstrap thresholds (eg, 1.5% distance and 98% bootstrap) have found recent infection as a predictor of membership in HIV transmission clusters (reviewed in [17]). Our data suggest that some of these results might have been affected by the strictness of the chosen thresholds, which inadvertently favored selection of recent transmission clusters over the chronic clusters.

This study has several limitations. One intrinsic challenge is that neither the timing nor the order of transmission events are strictly reflected in the pathogen phylogeny, which is also highly dependent on the sampling density of the target population [30]. However, the SHCS coverage of the Swiss HIV epidemic was estimated to be high [12], with >10 000 genotypic resistance tests done retrospectively using the SHCS biobank [11]. Moreover, 90 994 Los Alamos HIV-1 sequences were included to reduce the chances of random clustering.

Another limitation is that we were able to estimate the seroconversion date for only 29% of the cohort participants. This resulted in selection toward the MSM risk group, possibly because of the high rate of HIV testing, a key criterion in our estimation of seroconversion dates. We speculate that this selection toward MSM, combined with the high fraction of patients that were diagnosed while still at the recent phase, led to an overestimation of recent-phase transmission in our sample compared with the general Swiss HIV-positive population.

Finally, in contrast to the chronic-phase total VL, an accurate estimate of the total (AUC) recent-phase VL was not possible and was not incorporated into our statistical models, because most patients were enrolled in the cohort at variable times in relation to the acute-phase viremic peak. Despite these limitations, our work highlights the high fraction of recent-phase transmission and transmission during therapy interruptions, two key challenges for curbing HIV incidence with TasP.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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CHAPTER II

"Prescription of Postexposure Prophylaxis for HIV-1 in the Emergency Room: Correct Transmission Risk Assessment Remains Challenging"

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Description of personal contribution

The project was conceptualized by SKR, HH, AM and RDK. AM programmed the study database for systematic documentation of the cases. AM extracted the data, performed several rounds of quality control, and analyzed the data using various statistical tools, plots, and methods. AM produced all the tables and Figure 2 and 3. AM wrote the first manuscript draft together with HH. AM and SKR wrote the final version. AM and SKR revised the manuscript, AM performed additional analysis for the revision.

Research in context

When administrated fast enough – before 72 hours since exposure – highly active antiretroviral therapy can prevent the establishment of HIV infection. HIV post-exposure prophylaxis (PEP) after sexual exposure has been prescribed in resource-rich countries for almost two decades. However, it remains unclear whether the prescription practices reflect the risk of HIV transmission. In other words, it is not clear whether PEP is prescribed when the transmission risk is high and withhold when the risk is negligible. This issue is especially pertinent, as most PEP prescriptions take place in emergency room settings by physicians without specialized training in estimating HIV transmission risk.

To address this gap, we conducted a systematic evaluation of the adequacy of PEP prescription for all persons presenting at the emergency room of the Zurich University Hospital after consensual sex from 2007 to 2013. We first defined an epidemiology-based risk assessment algorithm, which incorporated the major risk factors for HIV transmission, including unprotected sex, HIV status of the source partner and high-risk transmission groups. Each visit was assessed with respect to the risk of HIV transmission and the actual PEP prescription decision, i.e. whether PEP was prescribed or not. We found that HIV PEP after a sexual incident was correctly prescribed in the majority of all visits (74%); however, in 10% it was not prescribed while indicated, and in 12% it was prescribed while no HIV transmission risk was evident. Both, patient demand or refusal and physician incorrect interpretation of the risk situation contributed to risk discordant decisions. In a multivariable analysis, the presence of the source partner (*i.e.* the partner with whom the sexual act took place) increased the odds of correctly withholding unnecessary PEP. We speculate that the findings presented in this work might have simple practical implications; in particular, emergency doctors may benefit from a PEP-related specialized HIV transmission risk recognition training.

Prescription of Postexposure Prophylaxis for HIV-1 in the Emergency Room: Correct Transmission Risk Assessment Remains Challenging

Alex Marzel, MSc,† Henriette Heinrich, MD,‡§ Lukas Schilliger, MSc,‡ Jan S. Fehr, MD,* Huldrych F. Günthard, MD,*† Roger Kouyos, PhD,*† and Silvana K. Rampini, MD‡*

Background: Limited data are available about the accuracy of postexposure prophylaxis (PEP) prescription in the emergency rooms. Here, we evaluated PEP prescription decision making with respect to the risk of sexual HIV transmission and the exposed person's fear vis-à-vis HIV.

Methods: Using a risk assessment algorithm, we retrospectively evaluated the adequacy of PEP prescription for all persons presenting at the emergency room of the University Hospital Zurich after consensual sex from 2007 to 2013. We used logistic regression to identify factors that correlate with risk-concordant and risk-discordant decisions.

Results: We documented 975 persons with a total of 1051 visits for PEP: 83% were men, 71% were Swiss, and 37% were men who have sex with men. In 74% of visits, the decisions were concordant with the risk evaluation algorithm (22% discordant, 4% unknown). In 61% (644/1051) PEP was prescribed; however, in 12% (76/644) the prescriptions were without indication of HIV transmission risk and were attributed to the exposed person's request. Importantly, in 10% (101/1051) of all visits, there were potential risks but PEP was not prescribed, either because of physician's decision or exposed person's refusal. The presence of the source partner strongly correlated with appropriately withholding PEP (adjusted odds ratio for giving PEP 0.05; 95% confidence interval: 0.03 to 0.08).

Conclusions: We found that 22% of PEP decisions were risk discordant because of exposed person's request, incorrect estimation of the sexual transmission risk by the physician, or exposed person's refusal to accept PEP. Emergency physicians may benefit from specialized risk assessment training and patients from education in HIV transmission risk awareness.

Key Words: postexposure prophylaxis, sexual risk, emergency room, decision making, HIV transmission, patient demand

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INTRODUCTION

Preventing HIV transmission is a major public health challenge.[1] Combined antiretroviral therapy (cART) is a valuable tool in this effort, either as preexposure (PrEP)[2] or postexposure prophylaxis (PEP).[3] PEP is highly effective in nonhuman primates in reducing the risk of simian immunodeficiency virus transmission by 77%–95%.[4] The main factors determining its effectiveness are the lag time between exposure and start of cART and its duration of intake.[4,5] In humans, PEP with Zidovudine alone reduced the risk of HIV transmission by 80% after occupational exposure.[6] In 1997, the Swiss Federal Office of Public Health (FOPH) recommended PEP after an HIV sexual risk exposure.[7]

After the introduction of cART by end of the 1990s, it was controversial whether PEP should be prescribed in nonoccupational (sexual) contacts.[8–10] Today, according to the European AIDS Clinical Society guideline[11] and the FOPH guideline updated in 2006,[12] PEP is primarily recommended for persons having unprotected sex (ie, anal, vaginal, or receptive oral sex with ejaculation) with a viremic HIV-positive partner or a partner with unknown serostatus but with the presence of HIV risk factors [ie, men who have sex with

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A.M. and H.H. both contributed equally. S.K.R., H.H., A.M., and R.K. designed the concept of the study. J.S.F. and H.F.G. gave critical input to the study protocol. L.S. and H.H. extracted all data from the charts. A.M. designed the database and performed the statistical analysis with the input of R.K. and S.K.R. A.M. and H.H. wrote the manuscript, and S.K.R. did the final editing. All authors read and revised the manuscript.

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men (MSM), sex workers, intravenous drug users (IDU), or persons from a country with a high HIV prevalence]. PEP is not recommended for persons having unprotected sex with an HIV-infected partner on successful cART. In all other situations with unprotected sex, individual risk should be evaluated. The internal guidelines from the University Hospital Zurich (USZ), relevant to the analyzed period, were largely derived from the FOPH guideline but left a lot of room for subjective risk evaluation by the physician, stating that:

> PEP should be prescribed in the following situations: unprotected vaginal, anal, or oral receptive intercourse with an HIV-positive partner or during menstruation. PEP should not be prescribed in cases of unprotected intercourse with a partner with unknown HIV status. In this case, fears and needs of the exposed person should be taken into account. Neither categorical nonprescription nor uncritical prescription can be encouraged.

Previous studies have already shown that the decision to use PEP is influenced by the experience of the physician in charge,[10] the emergency room (ER) setting,[8] and the exposed person's request.[10] Notwithstanding, people taking PEP frequently suffer from side effects that result in poor adherence, and as a result, only 65%–78% finish the 4-week PEP regimen.[13,14] Thus, the physician in charge must weigh the pros and cons of PEP carefully in each case.

Here we systematically assessed PEP decision making and factors that influence PEP decision making in daily practice in a large ER of a tertiary care hospital. Identifying these factors would be a major step in optimizing decision making. We retrospectively evaluated all persons seeking advice on PEP at the ER of the USZ between 2007 and 2013. We then reviewed whether the decision making was in agreement with a risk assessment algorithm for PEP prescription. In particular, we collected data on the demographic factors of the persons, the kind of sexual risk taken, the experience of the ER physician, and eventually the person's request and correlated these data with the evaluated decisions.

METHODS

Ethics

The study was approved by the Institutional Review Board of the USZ (KEK-ZH-Nr. 2013-0006).

Study Design

We identified, in a retrospective and cross-sectional manner, all persons admitted to the ER of the USZ seeking advice for HIV PEP in 2007–2013 by screening all electronic charts from that period for the following keywords: post-exposure prophylaxis, PEP, risk, exposure, sexual intercourse, and sex. Nonconsensual sex and occupational HIV exposure were excluded from the analysis. This ER has ~17,000 general internal medicine consultations per year.

Demographic Data and Sexual History

We collected the following data from the identified electronic charts for each exposed person: (1) demographic data, (2) a detailed sexual history, including type of sexual intercourse [ie, insertive, receptive, versatile (insertive and receptive), anal, vaginal, oral, smear of body fluids on healthy or wounded skin or mucous membranes, hand/feet to genitals contact, condom use, condom dysfunction], hours since exposure, or additional risk factors for HIV transmission (ie, menstruation, ejaculation, and sexually transmitted infections), and (3) the result from the HIV screening test at presentation. Based on the retrospective nature of the study, it was not possible to define oral sex with or without sperm exchange; thus, we considered unprotected oral sex as a risk situation. By default, every person seeking PEP at the ER should be tested for HIV on the spot. This screening was done with the fourth generation HIV antigen/antibody (Ag/Ab) combo screening test (Abbot, Wiesbaden, Germany), and its result should be available by 4 hours at the latest. Furthermore, for each source partner, we collected the gender, the risk group (ie, MSM, sex worker, from endemic country, IDU), last known HIV status, and the result of the HIV screening test performed at the ER if he presented together with the exposed person. If the presenting source partner was already known to be HIV positive, we extracted the last documented viral load value from his/her electronic chart. If no viral load was available, we requested an HIV-1 RNA viral load (HIV-1 Test, version 2.0; Roche, Branchburg). Eventually, we collected data on whether PEP was prescribed, the postgraduate education of the physician in charge, and the rational for the decision.

Risk Assessment Algorithm for PEP Decision Making

To assess the adequacy of PEP decision making, we developed an epidemiology-based risk assessment algorithm taking into account known risk factors for HIV transmission (Fig. 1). A time lag of >72 hours between sexual exposure and ER visit renders PEP inefficient, and in these cases, PEP was not indicated. Next, we ascertained the HIV status of the source partner, either by a negative HIV test done within the past 3 months or otherwise the result of an on-the-spot HIV test. In the case of HIV infection but an HIV RNA copy number <50/mL within the last 3 months or at presentation, we considered PEP as not indicated. The 3-month cutoff for having an HIV RNA below the detection limit is based on the 3-month intervals we see the HIV patients in our outpatient clinic, which is the standard of care. If the HIV status of the source partner was unknown, we classified the incident as low HIV transmission risk, and PEP was not indicated unless the source partner belonged to a high-risk group (Fig. 1).

We used this algorithm to categorize the PEP decisions as concordant (ie, "prescribed-and-indicated" or "not-prescribed-and-not-indicated") or discordant ("prescribed-while-not-indicated" or "not-prescribed-while-indicated").

Statistical Analyses

Bivariate P values for categorical variables were calculated using χ^2 test and Fisher exact test, and for

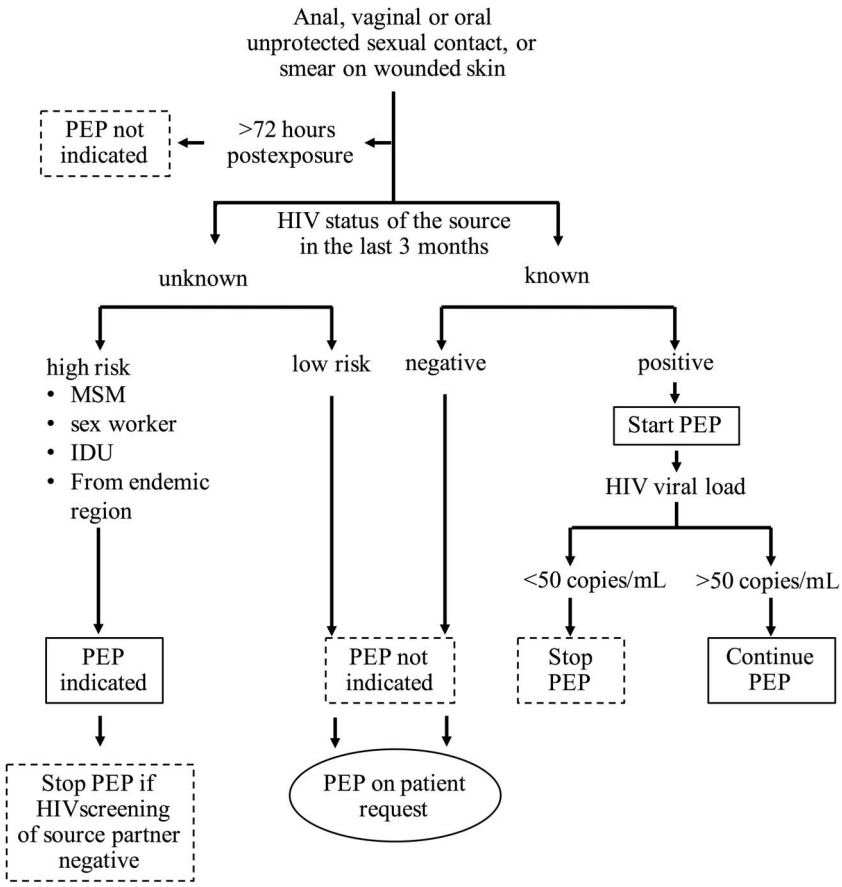

FIGURE 1. HIV transmission risk assessment algorithm used for the revision of PEP prescribed in the ER. All presentations after unprotected sexual intercourse (ie, condomless sex or condom dysfunction) were retrospectively evaluated using this risk assessment algorithm.

numerical variables, a Mann–Whitney *U* test was used. We used logistic regression to estimate correlating factors with prescribing or not prescribing PEP, stratified by concordant and discordant decisions (2 models). Statistical analysis was performed with R (version 3.2.3, http://cran.r-project.org).

RESULTS

Demographic Data of the Exposed Persons

Between 2007 and 2013, 975 persons visited the ER 1051 times to get PEP: 911/975 (93%) presented once and 64/975 (6.5%) presented repeatedly with 1 person presenting 7 times and a median interval of 1.7 years [interquartile range (IQR) 0.76–2.68] between the first and the last visit. MSM were overrepresented among those with more than one visit [37/64 (57%) vs. 315/911 (35%), Fisher exact test $P < 0.001$]. The number of visits remained stable over time with a median of 149 visits per year (IQR 133–162) (Fig. 2). The median age at first visit was 31 years (IQR 26–38). Out of all visits, 872/1051 (83%) exposed were men, 746/1051 (71%) were Swiss, 61/1051 (5.8%) were German, 42/1051 (4%) were Italian, and 202/1051 (19.2%) were from various countries. In 393/1051 (37.4%) visits, sexual contact between men was documented.

A large proportion of all PEP visits (43%, 451/1051) were on weekends (Table 1); 376/1051 (36%) visits were between noon and 6:00 PM and only 181/1051 (17%) were between midnight and 6:00 AM The median self-reported time lag between sexual intercourse and the ER visit was 20 hours (IQR 10–42), with 165/981 (17%) presenting after 48 hours and 46/981 (4.7%) after 72 hours (for 70 visits time since exposure was missing). MSM presented sooner after exposure than non-MSM [median 16 hours (IQR 5–32) vs. 24 hours (IQR 12–48), Mann–Whitney $P < 0.0001$] and also presented more on weekends [47% (185/393) vs. 40% (266/658), Fisher exact test $P = 0.04$].

In 4/1051 (0.4%) visits, the exposed person turned out to be HIV positive already at presentation.

Condom Use and Type of Sexual Intercourse

The exposed persons reported condomless sex in 527/1051 (50.1%) visits, condom breakage or slippage (condom dysfunction) in 433/1051 (41.2%) visits, and protected sex in 23/1051 (2.2%) visits. In 68/1051 (6.5%) visits, data were missing. MSM had mainly anal sex with 320/393 (81%) incidents. The anal sex was receptive in 120/320 (37%) visits, insertive in 84/320 (26%), versatile in 15/320 (5%), and data were missing in 101/320 (32%) visits. Heterosexual men and women had mainly vaginal intercourse with 387/448 (86%) and 153/176 (86%), respectively.

Demographic Data of the Source Partners

The source partner belonged to a group at high risk of being HIV infected in 670/1051 (63.7%) visits. More

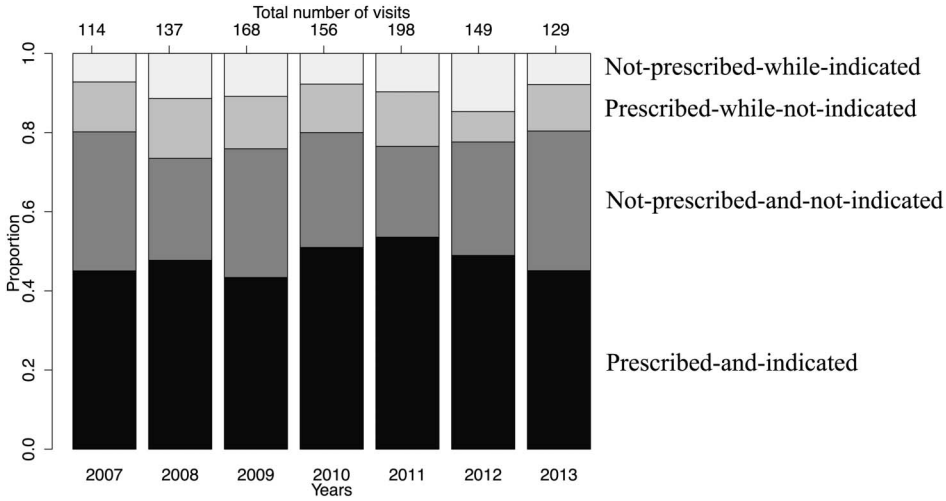

FIGURE 2. PEP visits to the ER of USZ by year and according to the categories defined by the risk assessment algorithm. The annual number of visits is shown on the *x* axis. *P* for trend 0.55. Forty-six visits with unknown category were excluded from the *y* axis for clarity.

specifically, 401/1051 (38%) were MSM, 256/1051 (24%) were sex workers, 46/1051 (4.3%) were from an HIV endemic region, and 11/1051 (1%) were IDU. The source partner belonged to more than one risk group in 41/1051 (4%) visits.

In 20% (211/1051) of the visits, the source partner did not belong to a risk group and has not presented, and his/her HIV status was unknown. This represents the fraction of low-risk presentations.

Source partners accompanied the exposed person in 170/1051 (16%) of the visits. However, we observed a decline in source partner presentation from 23% (27/114) in 2007 to 11.8% (15/129) in 2013 (P for trend = 0.042). Women were twice as likely to present with the source partner than men [27.9% (50/179) vs. 13.8% (120/872), Fisher exact test, $P < 0.001$]. The source partner was HIV infected in 131/1051 (12%) of the visits as self-reported or documented in his/her chart at the USZ. Out of those, for 60 (60/131, 45%), we could retrieve a viral load value, 23 were documented and within last 3 months of presentation (others were either self-reported or more than 3 months old). In 65% (39/60), the viral load was suppressed (<50 copies per milliliter), and for the rest, the median viral load was 3500 (IQR 456–10,000) copies per milliliter.

We did not observe an increase in sexual intercourse with HIV-infected source partners over time (P for trend 0.48).

Revision of the PEP Decision Making

PEP was prescribed in 644/1051 (61%) visits overall (Table 1). The PEP decision making of the physician in charge was in accord with the risk assessment algorithm in 779/1051 (74%) visits (ie, 485 prescribed-and-indicated and 294 not-prescribed-and-not-indicated) (Fig. 3). In 226/1051 (22%) visits, the decision making was discordant [ie, 125/226 (55%) prescribed-while-not-indicated and 101/226 (45%) not-prescribed-while-indicated] (see Table 1, Supplemental Digital Content, http://links.lww.com/QAI/A956).

The main reason for prescribing PEP when it was not indicated was the person's request in a low-risk situation in 76/125 (61%) visits. Although women made up only 78/644 (12%) of all prescribed PEPs, they were overrepresented with 27/76 (35%) in the category prescribed-while-not-indicated because of their request (Fisher exact test $P < 0.0001$). The remaining 49/125 (39%) PEPs prescribed in the category prescribed-while-not-indicated were explained by an incorrect interpretation of the sexual risk situation by physicians. Notably, in 485/644 (75%) visits, PEP was prescribed concordantly to the risk assessment algorithm.

Overall, there were 586 putative risk situations where PEP was indicated [ie, 485 prescribed-and-indicated (concordant) plus 101 not-prescribed-while-indicated (discordant)] (Fig. 3). In these 101/586 (17%) risk situations, PEP was not prescribed because of exposed person's refusal (31/101, 31%), the physicians not following the recommendation to give PEP within the lag time of 72 hours between the sexual incident and presentation (9/101, 9%), and the physician's incorrect interpretation of the sexual risk for HIV transmission (61/101, 60%). Notably, 20/61 (33%) sexual contacts were oral, and oral sex has a lower HIV transmission risk than vaginal or anal sex, especially if there is no exchange of sperm or blood. In 2/20 (10%) oral sex incidents, the source partners were HIV infected and one had 10^4 HIV RNA copies per milliliter in the blood. Infectious disease (ID) specialists took the decisions in 10/20 (50%) of these oral sex–only incidents, which is significantly more often than their overall involvement in decision making [179/1051 (17%), Fisher exact test $P < 0.001$]. Notwithstanding, in 41/61 incidents, a high risk for HIV transmission existed. Considering a total of 407 visits in which no PEP was prescribed, these 41/407 (10%) missed opportunities represent a considerable number in the context of HIV prevention. Finally, visits in which contact with a sex worker took place were significantly overrepresented in this category of not-prescribed-while-indicated (43.6%, 44/101 vs. 22.3%, 212/950, Fisher exact test $P < 0.001$).

We have not observed a change in the fraction of discordant decisions with time (Fig. 2). In the remaining 46/1051 (4%) visits, the data were too incomplete to categorize the decision making. The main missing variables were sex of the source partner (hence it was not clear if the exposed person belonged to the MSM risk group), condom use, and time since exposure or combination of those.

TABLE 1. Demographic Data and Sexual History Divided by PEP Prescription Outcome

	Overall	Not-prescribed	Prescribed	*P*
N	1051	407	644	
Age, median (IQR)	32.0 (26.0–38.0)	30.0 (26.0–37.0)	32.0 (27.0–38.0)	0.006
Sex, female, n (%)	179 (17.0)	101 (24.8)	78 (12.1)	<0.001
Swiss nationality, n (%)	746 (71.0)	278 (68.3)	468 (72.7)	0.147
MSM, n (%)	393 (37.4)	106 (26.0)	287 (44.6)	<0.001
>1 PEP visit, n (%)	140 (13.3)	38 (9.3)	102 (15.8)	0.003
Weekend, n (%)	451 (42.9)	175 (43.0)	276 (42.9)	1.000
Hours since exposure, median (IQR)	20.0 (10.0–42.0)	24.0 (10.0–49.0)	18.0 (10.0–38.0)	<0.001
Day time, n (%)				0.009
Midnight–06:00 AM	181 (17.2)	74 (18.2)	107 (16.6)	
06:00 AM–noon	182 (17.3)	51 (12.5)	131 (20.3)	
Noon–06:00 PM	376 (35.8)	148 (36.4)	228 (35.4)	
06:00 PM–midnight	312 (29.7)	134 (32.9)	178 (27.6)	
Year, n (%)				0.317
2007	114 (10.8)	48 (11.8)	66 (10.2)	
2008	137 (13.0)	50 (12.3)	87 (13.5)	
2009	168 (16.0)	72 (17.7)	96 (14.9)	
2010	156 (14.8)	57 (14.0)	99 (15.4)	
2011	198 (18.8)	64 (15.7)	134 (20.8)	
2012	149 (14.2)	64 (15.7)	85 (13.2)	
2013	129 (12.3)	52 (12.8)	77 (12.0)	
Condom, n (%)				0.001
Condom dysfunction	433 (41.2)	170 (41.8)	263 (40.8)	
Condomless sex	527 (50.1)	192 (47.2)	335 (52.0)	
With condom	23 (2.2)	18 (4.4)	5 (0.8)	
Unknown	68 (6.5)	27 (6.6)	41 (6.4)	
Type of intercourse, n (%)				
Anal	359 (34.2)	84 (20.6)	275 (42.7)	<0.001
Vaginal	543 (51.7)	244 (60.0)	299 (46.4)	<0.001
Oral	157 (14.9)	73 (17.9)	84 (13.0)	0.038
Only oral	94 (8.9)	52 (12.8)	42 (6.5)	0.001
Source partner risk group, n (%)				
MSM	401 (38.2)	107 (26.3)	294 (45.7)	<0.001
Sex worker*	256 (24.4)	74 (18.2)	182 (28.3)	<0.001
Endemic country	46 (4.4)	13 (3.2)	33 (5.1)	0.182
Intravenous drug user (IDU)	11 (1.0)	2 (0.5)	9 (1.4)	0.274
HIV status of the source partner, n (%)				<0.001
Negative	175 (16.7)	140 (34.4)	35 (5.4)	
Positive	131 (12.5)	16 (3.9)	115 (17.9)	
Unknown	745 (70.9)	251 (61.7)	494 (76.7)	
Source partner presented the same day, n (%)	170 (16.2)	138 (33.9)	32 (5.0)	<0.001
Deciding physician, n (%)				0.062
Resident in internal medicine	849 (80.8)	343 (84.3)	506 (78.6)	
ID specialist	179 (17.0)	58 (14.3)	121 (18.8)	
Internal medicine specialist	23 (2.2)	6 (1.5)	17 (2.6)	

*Sex work in Switzerland is legal and regulated.
†*P* values for categorical variables were calculated using χ^2 test; for age and hours since exposure, a Mann–Whitney *U* test was used.

Factors Correlating With PEP Decision Making

First, we used multivariable logistic regression to define factors that correlate with concordant decisions. Repeated visits to the ER for PEP [odds ratio (OR) 2.78; 95% confidence interval (CI): 1.54 to 5.03] and PEP decision making by ID specialists (OR 1.85; 95% CI: 1.09 to 3.12) were associated with concordant decisions on PEP prescription (ie, prescribed-and-indicated) and attendance of the source partner (OR 0.05; 95% CI: 0.03 to 0.08) and female sex (OR 0.16; 95% CI: 0.10 to 0.27) with concordant decisions on nonprescription (ie, not-prescribed-and-not-indicated) (Table 2).

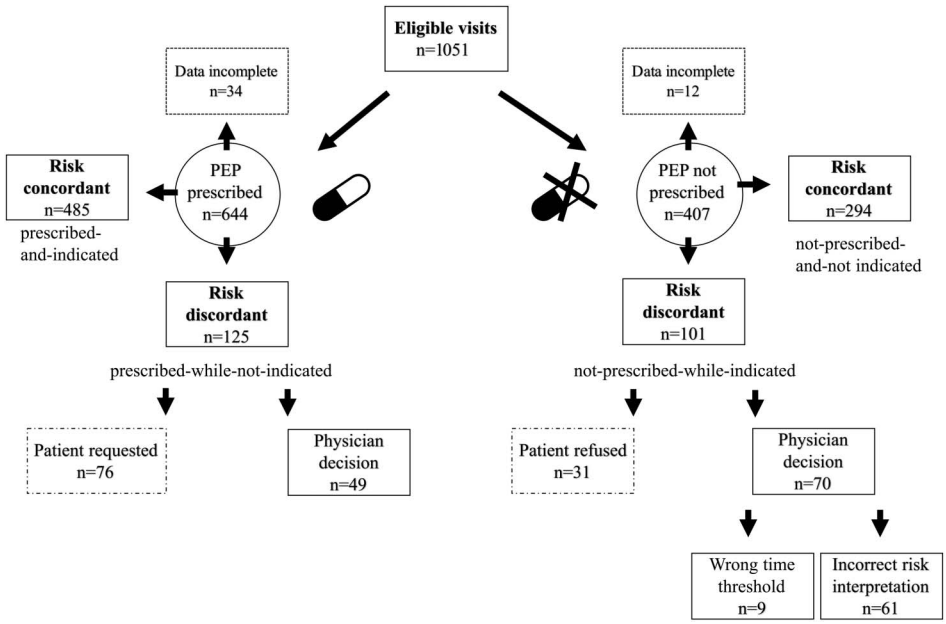

FIGURE 3. Flowchart of the study and the categories of PEP decisions.

In a second model, we used multivariable analysis to describe factors that might lead to discordant decisions. Female sex (OR 11.38; 95% CI: 4.10 to 31.6) and visits to the ER between 06:00 AM and noon (OR 2.92; 95% CI: 1.01 to 8.42) were associated with discordant decisions (ie, prescribed-while-not-indicated). Decision making by ID specialists as opposed to residents in internal medicine was associated with the decision category not-prescribed-while-indicated (OR 0. 38; 95% CI: 0.18 to 0. 81). This association became nonsignificant when the oral sex–only visits were excluded (OR 0.47; 95% CI: 0.20 to 1.07).

DISCUSSION

Accurately estimating the risk of HIV transmission after consensual sex and determining the necessity of PEP prescription are challenges for the ER physicians.[8] Here we retrospectively examined the accuracy of PEP prescriptions using a feasible risk assessment algorithm based on epidemiological HIV transmission risk and identified factors that correlate with risk-concordant decision making. Our main findings were that (1) the ER physician estimated the sexual risk for HIV transmission correctly in most visits (74%); however, in 10% of visits, PEP was not prescribed despite a risk situation. Furthermore, 12% of all PEP prescriptions were based on the exposed person's request rather than an appropriate indication. (2) Repeated visits and ID expert opinion were factors associated with correct prescription of PEPs, whereas consultation in the morning and female sex were associated with equivocal PEP prescriptions. (3) The presence of the source partner resulted in correctly withholding PEP.

Our risk assessment algorithm is in-line with the latest EACS and FOPH guidelines and was used to retrospectively define HIV transmission risk and prescription indications. The internal USZ guidelines, relevant to the analyzed period, did not specify the risk groups, hence giving leeway to the clinician in charge and requesting a detailed knowledge about HIV transmission risk. In addition, it was stated that the subjective fears and concerns of the exposed person should be taken into account, meaning that some PEPs were prescribed on demand in low-risk situations. Here, our aim was to estimate the objective risk of HIV transmission in each situation and to define factors correlating with correct and incorrect PEP prescriptions, not to describe the rate of adherence to internal guidelines.

We found that 12% of all PEPs were prescribed based on exposed person's request (prescribed-while-not-indicated). This might be because of inadequate counseling by the ER physician, limited knowledge of HIV transmission risk among them, or exposed person's demand because of fear of contracting HIV. Notably, patient request and uncertainty by the physicians affect drug prescription habits.[15,16] More women fell into this category; it can be hypothesized that women may be more concerned about HIV or simply look for maximum protection.[17] In addition, the gender of the physician in charge may influence the decision making, an issue not examined here.[18–21]

In 17% of the sexual incidents with a given risk for HIV transmission, PEP was not prescribed (not-prescribed-while-indicated). This was because of exposed person's refusal (n = 31) and physician's decision (n = 70). We speculate that balancing the actual risk of acquiring HIV against the potential side effects of PEP led to its refusal.[13,14] A word of caution must be added about the rather high number of physician decisions against PEP prescription: oral intercourse is a low-risk situation with an estimated HIV transmission rate of <4/10,000 incidents as opposed to insertive anal intercourse with 11/10,000.[22–24] We did not include such subtle distinctions in our risk assessment algorithm as ER physicians were not specifically trained to integrate the detailed sexual history into the assessment of the HIV transmission risk. In addition,

TABLE 2. Factors Correlating With PEP Decision Making

	Dependent Variable: PEP Prescribed (Yes/No)			
	Risk-Concordant Decisions		Risk-Discordant Decisions	
	Univariable, OR (95% CI)	Multivariable, OR (95% CI)	Univariable, OR (95% CI)	Multivariable, OR (95% CI)
Sex				
Men (ref)		1		1
Women	0.14 (0.09 to 0.22)*	0.16 (0.10 to 0.27)*	11.18 (4.24 to 29.48)*	11.38 (4.10 to 31.59)*
Age	1.03 (1.01 to 1.05)*	1.02 (0.99 to 1.04)	0.97 (0.94 to 1.01)	0.98 (0.94 to 1.01)
Nationality				
Non-Swiss (ref)		1		1
Swiss	1.30 (0.95 to 1.78)	1.01 (0.68 to 1.51)	1.04 (0.57 to 1.92)	1.65 (0.80 to 3.41)
More than one visit				
No (ref)		1		1
Yes	2.42 (1.50 to 3.91)*	2.78 (1.54 to 5.03)*	0.72 (0.30 to 1.70)	0.68 (0.26 to 1.83)
Postgraduate education of the physician in charge				
Internal medicine resident (ref)		1		1
ID specialist	2.28 (1.46 to 3.56)*	1.85 (1.09 to 3.12)†	0.48 (0.25 to 0.92)†	0.38 (0.18 to 0.81)†
Internal medicine specialist	4.17 (0.93 to 18.81)	3.32 (0.64 to 17.12)	0.22 (0.02 to 2.20)	0.27 (0.02 to 2.83)
Source presented with the exposed				
No (ref)		1		1
Yes	0.05 (0.03 to 0.09)*	0.05 (0.03 to 0.08)*	2.34 (0.72 to 7.58)	3.61 (0.92 to 14.12)
Presentation during weekend				
No (ref)		1		1
Yes	0.96 (0.71 to 1.28)	1.23 (0.85 to 1.78)	1.13 (0.65 to 1.94)	1.02 (0.55 to 1.92)
Time of presentation				
Midnight–06:00 AM (ref)		1		1
06:00 AM–noon	1.73 (1.04 to 2.90)†	1.65 (0.88 to 3.12)	1.76 (0.71 to 4.36)	2.92 (1.01 to 8.42)†
Noon–06:00 PM	0.98 (0.64 to 1.50)	0.94 (0.55 to 1.60)	1.33 (0.61 to 2.90)	1.77 (0.70 to 4.46)
06:00 PM–midnight	0.78 (0.51 to 1.21)	0.78 (0.45 to 1.35)	1.25 (0.56 to 2.78)	1.44 (0.56 to 3.68)
Year	1.03 (0.95 to 1.11)	0.93 (0.84 to 1.03)	0.92 (0.79 to 1.06)	0.94 (0.79 to 1.11)

Multivariable analysis using logistic regression of factors associated with prescription of PEP or not (binary dependent variable). Two separate models are shown, within the risk-concordant decisions (left) and within the risk-discordant decisions (right). Note that risk behavior was not included as a predictor because it is a major component of the outcome (used to classify decisions as justified or not).

*$P < 0.01$. All shown variables were included in the multivariable model.

†$P < 0.05$.

sexual history may be unreliable, and the notes in the charts are sometimes rudimentary, thus rendering them not useful for retrospective risk assessment. Finally, the local recommendations did not differentiate between sexual risks taken. That is why the ER physicians and, in particular, ID specialists might have advised against PEP after taking into account a low-risk situation (oral sex only), and this may explain the relatively high number of discordant decisions.

The presence of the source partner in the ER significantly improved the odds of concordant decision making. The immediate HIV testing of both sexual partners certainly defines the transmission risk in a best way. Importantly, current HIV tests are very sensitive and detect antigen and antibodies,[25] making it unlikely that an acute HIV infection is missed. Thus, persons seeking advice for PEP should be encouraged to present with their source partner[22] when receiving information about PEP.

Remarkably, the number of visits to our ER for PEP remained stable over the observation period. This can be explained by the interplay of the following factors. On the one hand, more risk behavior as the result of "the Swiss Statement" in 2008 that "HIV-positive persons on ART with undetectable viral loads and no other sexually transmitted infections may engage in condomless sex while in a stable relationship".[26–28] On the other hand, concern of an HIV infection may be less, leading to a smaller probability of demanding PEP when risk behavior occurred. This is backed up by recent studies showing an increase in unprotected sex in HIV-infected heterosexuals and MSM in both occasional and stable partnerships.[29] Alternatively, persons who engage in high-risk sexual activities and do not present to the ER may be ignorant about PEP and its benefit for reducing HIV transmission.[30] Indeed, knowledge of PEP was unexpectedly low (48.7%) even among HIV-infected individuals overall in the United Kingdom.[31] In Switzerland, even the long-lasting public health campaigns directed to HIV transmission prevention do not promote PEP. However, prescriptions doubled in a local gay health community center (www.mycheckpoint.

ch/en/zh, Bruggmann P, MD, written personal communication 06.01.2016) in the corresponding period. For some fraction of these, the prescribed PEP might have been obtained because of a claimed but nonexistent risk situation with the intention, on the recipient's side, to use it as PrEP.

We found that only 6.5% of all persons repetitively showed up at the ER for PEP. This number is substantially lower than the 20% reported in previous studies[32–35] and, thus, suggests that PEP is unlikely to promote higher sexual risk behavior, which is consistent with a report by Martin et al.[36] However, persons with high-risk sexual behavior, presenting repetitively for PEP, most likely would be the ideal candidates for HIV PrEP.

In summary, PEP decision making was adequate in the majority of visits; however, in every fifth visit, it was wrong. To benefit most from PEP, we see the need for further improvement in PEP decision making and counseling. Thus, ER physicians may benefit from a specialized risk assessment training that might incorporate the use of risk assessment algorithms. On the exposed person's side, future public health campaigns could increase PEP awareness alongside with knowledge about the risk situations that justify presentation. A special emphasis can be made on the benefits of presentation together with the source partner.

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CHAPTER III

"*Mining for pairs: shared clinic visit dates identify steady HIV-positive partnerships"*

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Description of personal contribution

The project was conceptualized by RDK, AM and HFG. AM extracted the data from the SHCS patient and resistance databases, performed quality control, constructed the phylogenetic trees, and analyzed the data using various statistical tools, plots, and methods. AM wrote the R package with contribution of TT. AM and RDK wrote the first manuscript draft and the final version.

Research in context

In this work, we propose and validate an interdisciplinary computational data mining approach for the detection of steady transmission and serosorting HIV infected couples using shared clinic visit dates. The ability to characterize these pairs is a long-lasting methodological challenge, which if solved, will assist targeted prevention of HIV transmission and identification of scarce pairs for biological research, when a biobank is available. This can help to address pertinent research questions on within-pair quasi-species exchange, transmitted drug resistance and viral recombination on the biological level and targeted prevention on the public health level. Moreover, the proposed method can serve as an additional confirmation criterion for increasingly used phylogenetically linked transmission pairs.

Currently, the stable transmission and serosorting pairs that can be detected with our method are in very high demand both for biological and epidemiological research. Moreover, in addition to research questions that are centered around HIV biology and epidemiology, the detected pairs can shed light on within pair transmission of other common STIs (*i.e.* syphilis). Importantly, our method has a potential for broader application by users, beyond the HIV field. It is applicable to any type of observational epidemiological data containing visit dates. Almost all epidemiological studies ignore the social network underlying their study population and must therefore, by necessity, assume that this population is composed of independent individuals. Our method allows to recover some of the strongest links in these networks, which will enable to study key questions that cannot be addressed in the classical "independent individuals" paradigm (e.g. infectious diseases transmission, risk clustering, etc.).

We implemented the proposed method demonstrating its usefulness and applicability using the Swiss HIV Cohort Study as a showcase. Implementing the proposed concept allowed us to gain new insights about an epidemiologically relevant subpopulation of steady HIV positive transmission and serosorting couples of mixed ethnicity with large within-pair age gaps (and the man being older and white). These pairs were validated by three independent criteria:

an extensive phylogenetic tree, self-reported steady HIV positive partnership, and HIV risk group affiliation.

With 39 cohorts of HIV infected individuals, from Europe alone, and large US cohorts (i.e. MACS) alongside with large consortiums (i.e. IeDEA), we speculate that this simple method might have application opportunities beyond the Swiss HIV Cohort Study.

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ORIGINAL RESEARCH

Mining for pairs: shared clinic visit dates identify steady HIV-positive partnerships

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Objectives

Here we examined the hypothesis that some stable HIV-infected partnerships can be found in cohort studies, as the patients frequently attend the clinic visits together.

Methods

Using mathematical approximations and shuffling to derive the probabilities of sharing a given number of visits by chance, we identified and validated couples that may represent either transmission pairs or serosorting couples in a stable relationship.

Results

We analysed 434 432 visits for 16 139 Swiss HIV Cohort Study patients from 1990 to 2014. For 89 pairs, the number of shared visits exceeded the number expected. Of these, 33 transmission pairs were confirmed on the basis of three criteria: an extensive phylogenetic tree, a self-reported steady HIV-positive partnership, and risk group affiliation. Notably, 12 of the validated transmission pairs (36%; 12 of 33) were of a mixed ethnicity with a large median age gap [17.5 years; interquartile range (IQR) 11.8–22 years] and these patients harboured HIV-1 of predominantly non-B subtypes, suggesting imported infections.

Conclusions

In the context of the surge in research interest in HIV transmission pairs, this simple method widens the horizons of research on within-pair quasi-species exchange, transmitted drug resistance and viral recombination at the biological level and targeted prevention at the public health level.

Keywords: cohort studies, data mining, epidemiology, HIV, phylogeny, transmission

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Introduction

Identifying and characterizing steady - as opposed to occasional - HIV transmission pairs is a key challenge in HIV epidemiology and prevention, especially in light of data that suggest that steady partners are an increasing source of HIV infection with increasing age among men who have sex with men (MSM) [1,2].

In this work, we proposed and validated a new approach for identifying steady HIV transmission pairs and steady serosorting couples, that is, HIV-positive individuals who are not infected by the same viral variant but prefer other HIV-positive individuals as sexual partners. We examine the simple hypothesis that some steady

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*See Appendix.

partners can be identified in longitudinal cohorts, because they attend the follow-up and the laboratory visits together. This is likely to be reflected in the number of shared follow-up visits, with a frequency that is unlikely to be attributed to chance.

Identifying and characterizing HIV-positive steady transmission pairs has the potential to shed light on their demographic and epidemiological features, which are not likely to change after they become seroconcordant. Knowing these characteristics can facilitate targeted prevention of HIV transmission. Moreover, identification of steady HIV-positive pairs that engage in unprotected sex, given a still high prevalence of late treatment initiation and treatment interruptions [3,4], can widen the horizons of research on within-pair quasi-species exchange, transmitted drug resistance and viral recombination [5]. In addition to transmission pairs, the proposed method can identify steady serosorting couples. Detailed longitudinal data on these pairs are very scarce.

We used the Swiss HIV Cohort Study (SHCS) as a showcase for the applicability of our method as the SHCS is renowned for its breadth and depth of demographic and molecular data as well as for its high coverage of the HIV-positive population in Switzerland [6]. However, with 39 cohorts of HIV-infected individuals from Europe alone [7], and large ongoing US cohorts [8], this method is expected to have ample application opportunities.

Implementing the proposed concept allowed us to identify an epidemiologically relevant subpopulation of steady HIV-positive transmission pairs and serosorting couples of mixed ethnicity with large age gaps. Importantly, at the meta-level, our study demonstrates how a detailed, prospective, and de-identified data set can be used - if publicly available and in the wrong hands - to obtain sensitive information at the level of individual patients.

Methods

Study population: the Swiss HIV Cohort Study and the drug resistance database (SHCS-DRDB)

The SHCS is a large, prospective, multicentred, interdisciplinary study established in 1988. It is highly representative of the HIV epidemic in Switzerland, with an estimated coverage of at least 45% of all HIV-infected individuals, 69% of all AIDS patients in Switzerland and 72% of all individuals treated with antiretroviral therapy (ART) [6,9].

During the bi-annual follow-up visits, clinical and demographical data are collected for each participant using the study questionnaire, which includes a question about being in a steady partnership with another HIV-positive individual (without asking about the identity of the partner). In addition, the standard of care requires monitoring of viral load (since 1996) and CD4 count, every 3 months. In practice, every second visit is a combined cohort follow-up and laboratory monitoring visit; thus, on average, a patient is expected to have a total of at least four visits per year. Ideally, the visit dates are chosen by the patients without restrictions. The drug resistance database contains HIV-1 polymerase (pol) sequences for ~60% of all patients ever included in the cohort and ~85% of patients enrolled after 1996 [10]. The cohort database is de-identified.

Identification of steady transmission pairs and serosorting couples using shared follow-up visits

Our method is based on the simple intuition that pairs of patients who share a larger number of visits than expected by chance are more likely to represent pairs who coordinate their visits to the clinics, which might reflect being either steady transmission pairs or serosorting couples. In its simplest version, this algorithm would use the number of shared visit dates within a couple as the test statistic. The threshold number of shared visits that can be expected by chance is determined by randomly shuffling cohort visits across individuals and counting the number of random visit collisions (a further explanation is given below). Pairs exceeding that threshold are flagged as candidate pairs for further evaluation and validation.

Below, we present a slight addition to this algorithm which also adjusts for the length of follow-up.

Briefly, for each patient who consistently visited only one of the seven SHCS study centres, all the shared visits with the other eligible patients from the same centre were extracted. To remove a potential bias stemming from a nonrandom choice of visit dates as a consequence of a wish to consistently visit a certain physician, we counted for each physician the median interval between the dates he/she sees patients. Visits of physicians who saw patients infrequently were excluded if a median time interval between the dates on which they saw SHCS patients exceeded 7 days. As these physicians have a large time span between visits, several patients might choose the days these physicians work and share large numbers of visits without actually being a pair.

Adjustment for the total number of visits per pair by a penalty term

The chances of two unrelated cohort members sharing a visit increase with the overall number of visits of each member of the pair. To account for this, we adjusted the number of shared visits per pair using the following

intuition: visits occur typically each quarter and a quarter has approximately 75 eligible days for visits. If we make the simplifying assumption that all visits for an individual with the lower number of visits within a pair fall within quarters where the other individual also has a visit and that the visits are distributed uniformly and independently across the 75 eligible dates of each quarter (this parameter should be tailored to each cohort; see Supporting Information S1 for sensitivity analysis with a 30-day window), then the probability of obtaining S shared visit dates per randomly chosen pair can be approximated as:

$$p_{\text{shared}}(S, T_a, T_b) = \binom{\min(T_a, T_b)}{S} \left(\frac{1}{75}\right)^S \left(1 - \frac{1}{75}\right)^{\min(T_a, T_b) - S}, \quad (1)$$

where T_a and T_b are the total number of visits made by patients a and b, respectively, and S is the number of shared visits. p_{shared} can now be used to identify pairs of patients whose number of shared visits is very unlikely to occur by chance (by determining a threshold and considering all pairs with p_{shared} below this threshold as potential transmission or serosorting pairs; see the Bonferroni-corrected method below) and takes into account the effect of unequal visit numbers on the probability of sharing visits. As our main concern is false positives (artificially low p_{shared}) and the last factor is expected to be large, we assume that:

$$\tilde{p}_{\text{shared}}(S, T_a, T_b) = \binom{\min(T_a, T_b)}{S} \left(\frac{1}{75}\right)^S. \quad (2)$$

Finally, we consider instead of $\tilde{p}_{\text{shared}}$ the equivalent quantity S' (Eqn. 3). Equivalence here means that selecting potential stable transmission and serosorting pairs as those whose $\tilde{p}_{\text{shared}}$ is lower than a threshold $\tilde{p}_{\text{thr}}$ yields the same pairs as selecting those pairs whose S' is above a threshold S'_{thr}, where $S'_{\text{thr}} = -\log(\tilde{p}_{\text{thr}})/\log(75)$:

$$S' = -\frac{\log(\tilde{p}_{\text{shared}})}{\log(75)} = S - \frac{\log\left[\binom{\min(T_a, T_b)}{S}\right]}{\log(75)} \quad (3)$$

S' can be interpreted as an effective (adjusted) number of shared visits penalized by the term:

$$\frac{\log\left[\binom{\min(T_a, T_b)}{S}\right]}{\log(75)} \quad (4)$$

This accounts for the varying background probability of shared visits as reflected in the varying length of follow-up. Note that $\min(T_a,T_b)$ ensures a differential penalty such that if $S = \min(T_a,T_b)$ the penalty is zero and the larger the $\min(T_a,T_b) - S$ the larger the penalty, reflecting the intuition that if all the visits of one member of the pair are shared with the other [hence $S = \min(T_a, T_b)$], then there is a higher probability that such a pair is related (and hence should not be penalized).

Determining the threshold for the number of shared visits using shuffling

As a consequence of the multiple comparisons problem (3 029 261 pairs were analysed) and strongly simplifying assumptions about the uniform distribution of visits, the above expressions cannot be used to directly derive threshold values for the effective number of shared visits S'. Hence, we used the following shuffling strategy to derive the null distribution. For each of the seven study centres, the visits were shuffled within each quarter (such that the original distribution of the number of visits per individual was preserved) and the number of randomly collided shared visits per pair was counted and penalized using Eqn. 3. In other words, the observed visit dates from a given quarter were randomly reassigned between the patients who attended during this quarter (see Supporting Information S1 for shuffling within a 30-day window). The shuffling procedure was replicated 100 times and allowed us to estimate the background number of shared visits that are expected to occur by chance for each centre (Fig. 1). From these shuffled data sets, we estimated the fraction of pairs that were expected to be falsely identified in our data set as transmission/serosorting pairs for a given threshold S'_{thr}, denoted $F_{FP}(S'_{\text{thr}})$, as the maximum number of pairs with $(S'_{\text{shuffled}} \geq S'_{\text{thr}})/(S'_{\text{unshuffled}} \geq S'_{\text{thr}})$. We then chose the lowest threshold S'_{thr} such that the fraction $F_{FP}(S'_{\text{thr}})$ is $< 1\%$ for further analysis.

Alternative method: determining the threshold for the number of shared visits using Bonferroni correction

An alternative and less computationally demanding approach for the detection of possible steady pairs would be calculating the probability of sharing a given number of visits (p_{shared}) using Equation 1, and then determining the selection cut-off using Bonferroni correction [11] for multiple comparisons [Type I error alpha (0.01)]/[number of pairs that shared at least one visit (3 029 261)]. Pairs with p_{shared} below the threshold were flagged for further evaluation. We implemented both approaches (shuffling and Bonferroni correction) and compared their performances.

Phylogenetic tree and genetic distances

We used phylogenetic linkage as a key criterion for the validation of the putative steady transmission pairs that were detected using this method. A total of 19 893 partial HIV-1 pol sequences from 10 970 SHCS cohort participants (years 1989–2015) were pooled with 116 408 background, non-

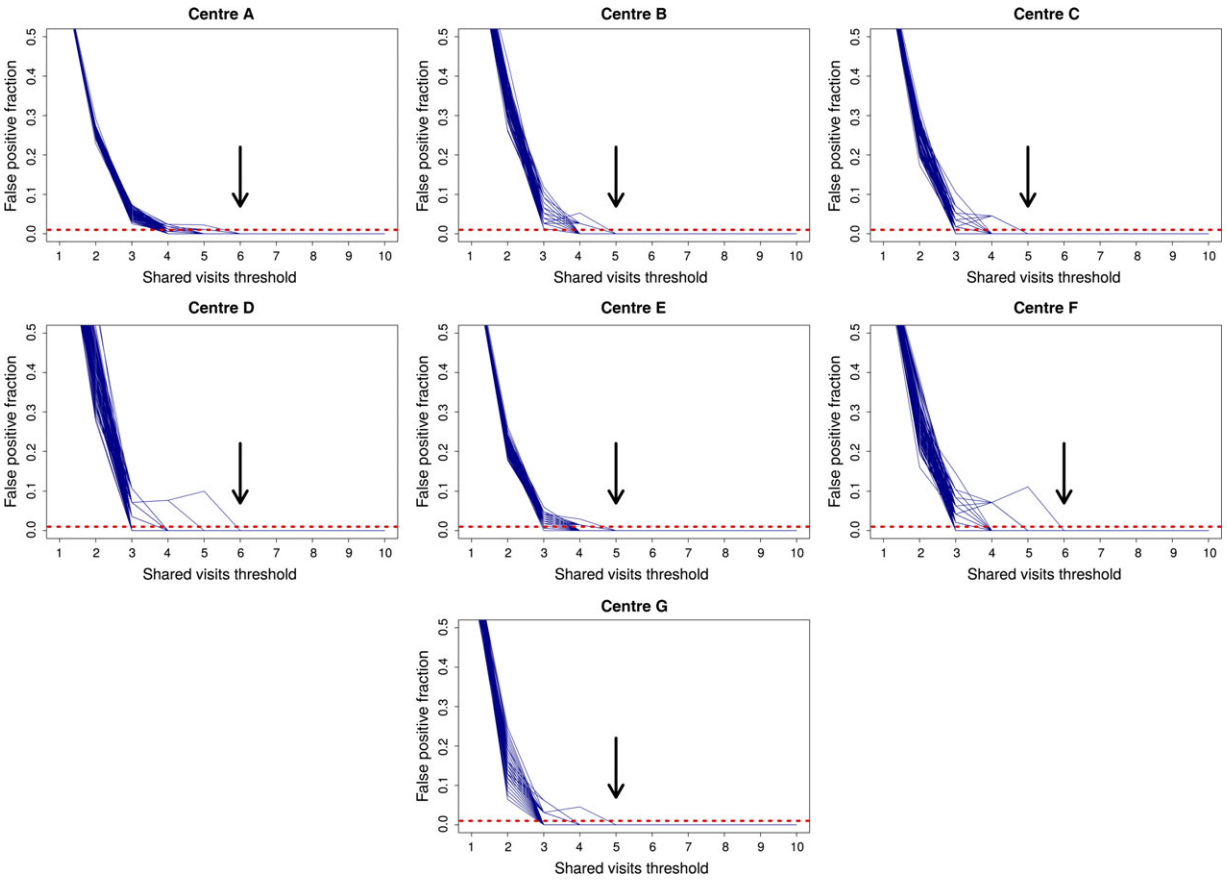

Fig. 1 Shuffling simulations for the determination of the background probability of sharing a follow-up visit in seven Swiss HIV Cohort Study (SHCS) centres (labelled a–g). Each simulation is depicted by a blue line; the thresholds (S'_{thr}) used are shown on the *x*-axis and the minimal false positive fraction on the *y*-axis. The 1% theoretical false positive threshold is depicted by the horizontal red dashed line. The lowest threshold that is below the 1% cut-off for each centre is indicated by an arrow.

Swiss HIV-1 pol sequences from the Los Alamos database for a large phylogenetic tree constructed using FastTree [12]. See Supporting Information S1 for technical details.

Ethics

Ethical approval of the SHCS and written informed consent from all participants were obtained.

Statistical analysis

Statistical analysis was performed using R (v 3.2.3) [13,14] with some functions written in C++ to improve performance [15].

R package

An R package named *svisits* was constructed to facilitate easy application (available at https://github.com/alexmarzel/svisits). A short vignette that demonstrates the package application can be found in Supporting Information S2. A data set with visit dates that were simulated (to protect our patients' privacy; see Discussion) using the empirical distributions in the SHCS is embedded in the package.

Results

Data description

We analysed data for 16 139 SHCS patients, accounting for a total of 434 432 visits from 1990 to 2014. A total of 3 029 261 pairs of patients who attended the same clinic on the same day at least once were identified across all seven centres. For those pairs, the number of unadjusted shared visits per pair ranged between 1 and 72, with a median of 1 (IQR: 1–2). This indicates that sharing more than one visit with another patient was rare. Following the shuffling and the correction penalty,

the 1% false-positive thresholds for each centre ranged from 5 to 6 effective shared visits per pair (Fig. 1), depending on the centre.

Method validation

In line with our assumption, the number of eligible days for a visit did not change substantially during the follow-up time, with a median of 62 (IQR: 55–65) days per quarter. In 192 out of 3 029 261 pairs, the number of adjusted shared visits exceeded the established thresholds S'_{thr}; these pairs were selected for further evaluation. Two pairs comprised of heterosexual women were excluded as implausible transmission pairs. The remaining pairs were validated as potential transmission pairs using three criteria: (i) monophyletic clustering on the extensive (136 301 tips) phylogenetic tree and a maximal genetic distance of 2.5% between sequences; (ii) a self-report of having an HIV-positive steady partner by both members of the pair during the period between the first and the last shared visits with the other member (question introduced in the SHCS questionnaire in the year 2000); (iii) belonging to the same HIV transmission risk group (MSM, heterosexual or injecting drug use).

Of the identified pairs, 89 had available data for all three validation criteria. Of these, 50 clustered on the phylogeny and were below the genetic distance cut-off. Thirty-three pairs (33 of 89; 37%) were confirmed using all three criteria (Fig. 2). The median pairwise genetic distance of the viruses from these 33 pairs was low, 0.5% (IQR: 0.15–0.98%), supporting the conclusion that these were HIV transmission pairs [16,17]. Additionally, the Shimodaira–Hasegawa local node support values for the most recent common ancestor (MRCA) node between the two pair members were also high, with a median of 97% (IQR: 92–99%), suggesting a high phylogenetic certainty of the monophyletic clusters.

Eight pairs were not linked by the phylogeny but were risk group concordant and declared having an HIV-positive steady partner. These pairs, who did not share a genetically similar clone of the virus (median genetic distance 8.5%; IQR: 3.5–10%) but had many shared visits (median 13; IQR: 8–23; adjusted), might represent not transmission but steady serosorting couples (Fig. 2); however, these pairs require stricter validation (Supporting Information S1). Fourteen of 89 (15.7%) pairs were discrepant by all three criteria and can be attributed to a residual nonrandom clustering of visit dates which was not efficiently accounted for by shuffling as a consequence of latent nonrandom visiting patterns.

Importantly, the 33 pairs validated by all three criteria had a significantly higher median number of effective

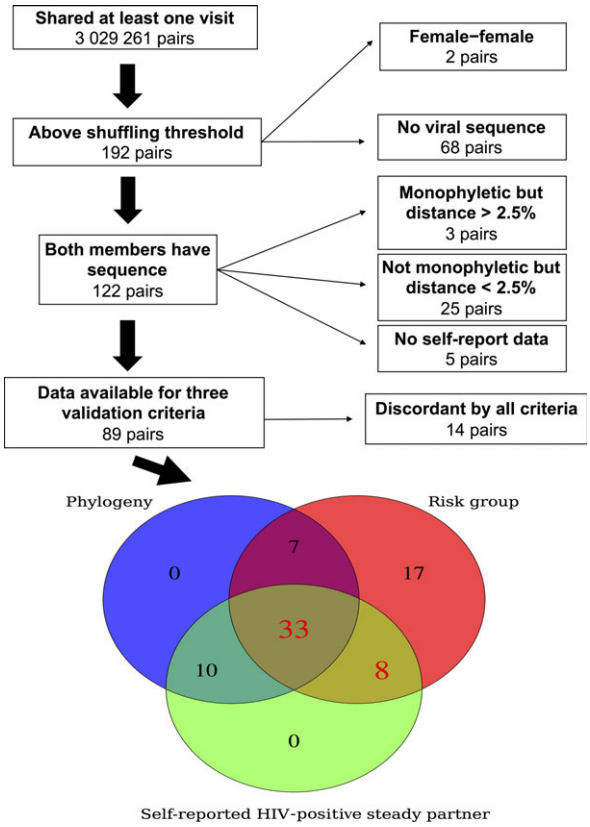

Fig. 2 Flow chart and Venn diagram (bottom) demonstrating the validation of 75 putative pairs detected using shared cohort follow-up visit dates. Red numbers indicate putative transmission (n = 33) and serosorting (n = 8) pairs.

shared visits in comparison to pairs that were not validated by any of the criteria; 16 versus 6, respectively (Wilcoxon $P < 0.0001$; P for trend = 0.044), which supports our basic concept that a high number of effective shared visits is indicative of a putative transmission pair (Fig. 3, top). Moreover, we also noticed that the pairs that were validated by all criteria had a significantly lower median number of total visits per pair ($T_a + T_b$) than unvalidated pairs (64 versus 153, respectively; Wilcoxon $P < 0.0001$; P for trend = 0.047; Fig. 3, bottom). This indicates that the latter pairs were probably false positive because of the overall very high number of visits per pair, despite the adjustment for the total follow-up time per pair (which is effective but not perfect).

Finally, we explored whether there was a negative association between the effective (penalized) and unadjusted number of shared visits and the minimal genetic distance within each pair, among the 89 pairs eligible for validation. For the adjusted visits, the Spearman's rho was −0.35 ($P < 0.0001$), while the association for unadjusted visits was weaker and nonsignificant, −0.17

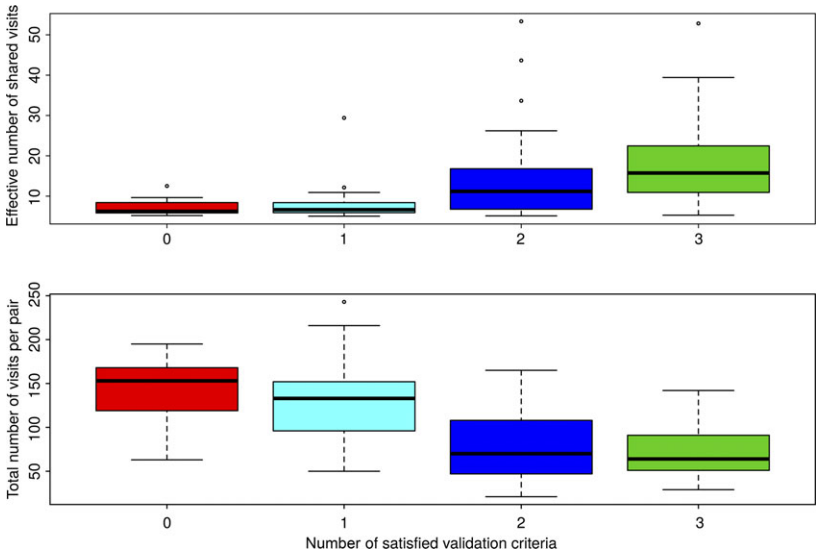

Fig. 3 Relationship between the number of validation criteria that were met by the putative steady partnerships (*x*-axis) and the effective number of shared visits per pair (*y*-axis, top), as well as the joint number of visits made by both pair members (*y*-axis, bottom).

($P = 0.11$), further supporting both our basic assumption concerning the correlation between the number of shared visits and being a possible transmission pair (i.e. more shared visits, the closer are the viruses) and the necessity of penalizing the total follow-up time per pair.

As an alternative, faster set-up, applying the Bonferroni-corrected threshold provided qualitatively equivalent results with a total yield of 271 candidate pairs (Fig. 4). The overlap between the two approaches was high: 192 pairs. Validation analysis revealed the same 33 transmission pairs (validated by all three criteria) as found using the shuffling, plus three additional pairs - adding face validity to our study - as well as the same eight serosorting pairs plus one. However, this approach had a slightly higher false-positive rate; of the 132 of 271 pairs that had available data for validation, 23% (31 of 132) were not validated by any of the three criteria as compared with 16% false positives using the shuffling (not significant; $P = 0.16$) Chi-squared test.

Characterization of steady transmission pairs

Following the detection and the validation of the 33 potential steady transmission pairs, we went on to characterize their main features. Twenty-six pairs (79%; 26 of 33) were heterosexual and seven pairs were MSM.

In 15 pairs both members were of white ethnicity, five pairs were concordantly of black ethnicity and one pair was concordantly Asian. Twelve pairs (36%; 12 of 33) were of mixed ethnicity; ten were white–Asian (eight were heterosexual, with the man always being white, and two were MSM pairs). The two remaining inter-ethnic pairs consisted of a white man and a Latino woman. Pairwise comparisons showed that white–Asian pairs had a significantly higher mean age gap in comparison to white–white and black–black pairs [19 years versus 6.7 years ($P = 0.002$) and 5.6 years ($P = 0.025$), respectively; Tukey and Kramer (Nemenyi) test [18]; Fig. 5].

Among the ten white–Asian couples, eight had virus of subtype CRF01_AE, one virus of subtype 02_AG, and one virus of subtype B, while among the white–white pairs, the majority (73%; 11 of 15) had virus of subtype B (Fisher's exact test; $P = 0.002$).

Next, we examined whether these steady pairs used condoms during the period between the first and last shared visits. We found that 27 of 33 (82%) reported condomless sex with a steady partner during the assumed steady partnership period. Notably, seven births were recorded by seven different women. In six of these seven cases, the man declared that he had fathered a child during the corresponding periods, which further adds to the validity of our definition of the pairs as steady, as it is well documented in sociological research that childbearing decreases the probability of union dissolution [19].

Discussion

We have presented an epidemiological data mining approach to identify putative transmission pairs or serosorting couples based on shared visit dates in observational cohorts. There has been a growing use of HIV

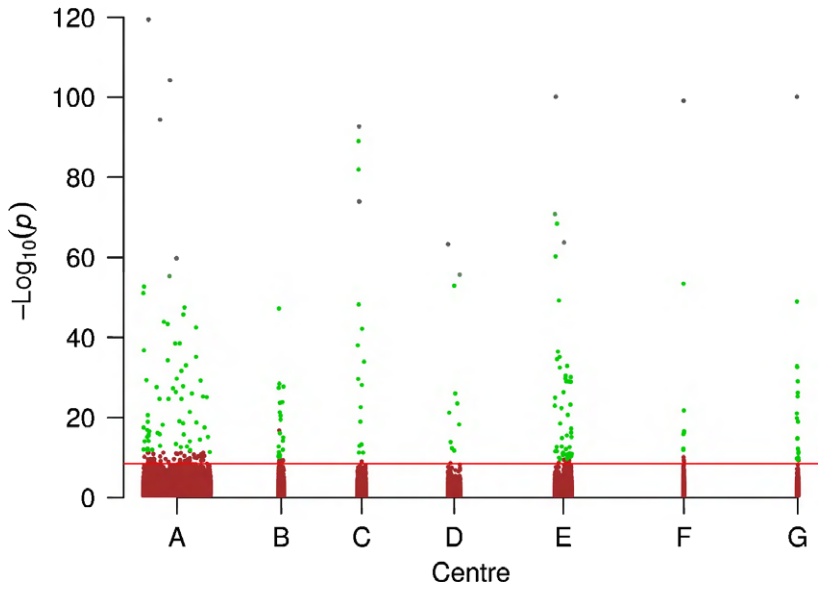

Fig. 4 Probabilities of the observed number of shared visits for each pair with at least one shared visit, as determined by Equation 1, stratified by Swiss HIV Cohort Study (SHCS) centre (labelled a–g; *x*-axis); values are $-\log_{10}$ transformed. The horizontal red line shows the Bonferroni-corrected alpha (3.301135e-09); pairs below this threshold (i.e. above the line) were chosen for further evaluation as possible steady transmission pairs or serosorting couples. Green circles designate the pairs flagged by the shuffling method.

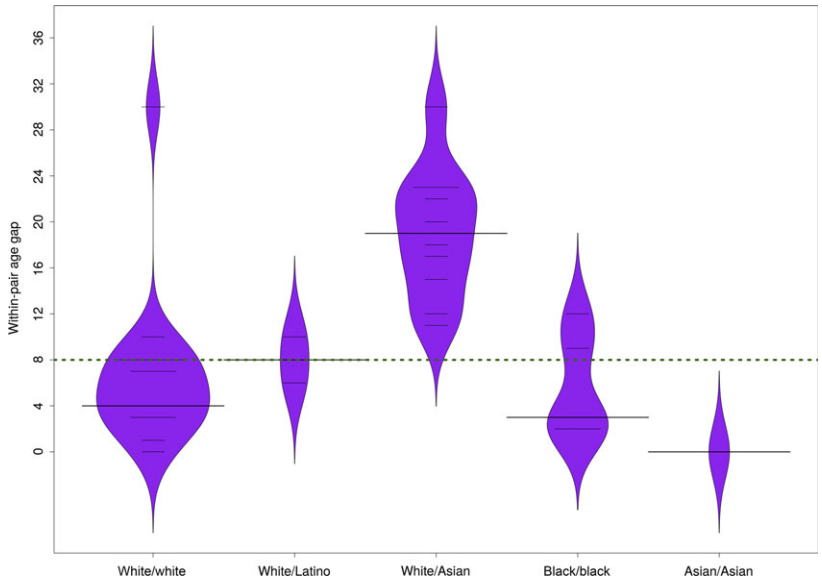

Fig. 5 Beanplot showing the within-pair age differences (in years) between the five observed combinations of ethnicity of the 33 validated steady transmission pairs. The dashed green line shows the pooled median of the entire sample.

resistance test sequences for epidemiological linkage of transmission pairs, which has led to important insights into key questions in HIV epidemiology [3,17,20–22]. However, it is also clear that phylogenetic linkage alone cannot prove transmission [23–25]. This is mainly because the validity of the phylogenetic clustering is highly dependent on the sampling density of the target population [24]. Our approach has the advantage of detecting putative transmission pairs first based on an aberrant number of shared visits, then validating them

using phylogeny or other available criteria, hence without putting the entire weight of evidence on the phylogeny.

As is often the case in data mining, the main usefulness of our method lies not in its predictive accuracy, but in its ability to narrow the screened population from millions of possible combinations to several dozen candidate pairs that are to be assessed in depth and validated, as screening and validation of all the possible combinations are otherwise impractical. Hence, the method is mainly useful as an *a priori* filtering tool with a good input-to-output ratio. By the same token, classical epidemiological concepts such as positive predictive value and receiver operating characteristic (ROC) are not applicable to this problem, because the real structure of the transmission chains is mostly unknown [23–25].

We refrain from arguing that pairs that share clinic visits are representative of all HIV-positive steady pairs. Yet, even if these pairs are selective, this does not nullify the biological level insights that can be gained by using their samples. As early treatment initiation is now universally recommended regardless of CD4 count [26], a method for retrospective detection of transmission pairs that is based on samples that have already been collected and stored is timely.

We found eight heterosexual, mixed-ethnicity steady transmission pairs with subtype CRF01_AE. This result is in line with a recently published analysis of the global dispersal patterns of this subtype which is prevalent in Asia, predominantly in Thailand. Angelis *et al.* [27] showed that Switzerland experienced at least 15 heterosexually driven migration events of CRF01_AE, which is larger than expected and the highest number in continental Europe. Our study suggests that steady, mixed-ethnicity partnerships with a large age gap (with the man being white and older) contribute to these introduction events. This observation opens up a targeted prevention opportunity.

Apart from epidemiological level insights, identification of steady transmission and serosorting pairs can widen the horizons of research at the biological level, providing insights into problems that are otherwise difficult to tackle; for example: (i) prospective viral quasi-species exchange between the steady partnership members (both transmission pairs and serosorting couples), given the fact that treatment interruption is still prevalent [3]; (ii) exchange of transmitter-founder strains and prospective viral diversification, and (iii) within-pair transmission of drug resistance. In addition to research questions centred around HIV biology and epidemiology, the detected pairs can shed light on within-pair transmission of other common sexually transmitted infections (e.g. syphilis) which are currently on the rise in the HIV-positive population [28,29].

Our study also demonstrates how even de-identified data, when aggregated longitudinally and in great detail, can still be used to obtain information about individuals and their health, sexual patterns and habits. In the era of 'big data' and its increased - and as some argue inevitable [30] - use in public health research, our study emphasizes that greater effort should be made to protect the privacy of the patients and that making anonymized health data publicly available might lead to unexpected consequences [31]. Similar privacy concerns, but in the context of the deposition of anonymized sequencing data sets, were raised by Gymrek *et al.* [32] who were able to successfully identify personal genomes by combining anonymized metadata with Y-chromosome haplotypes.

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Conflicts of interest: HFG has been an adviser to and/or consultant for the following companies: GlaxoSmithKline, Abbott, Gilead, Novartis, Boehringer Ingelheim, Roche, Tibotec, Pfizer and Bristol-Myers Squibb, and has received unrestricted research and educational grants

from Roche, Abbott, Bristol-Myers Squibb, Gilead, AstraZeneca, GlaxoSmithKline, and Merck Sharp & Dohme (all money went to the institution). EB has been a consultant for BMS, Gilead, ViiV Healthcare, Pfizer, MSD and Janssen. He has received unrestricted research grants from Gilead, Abbott, Roche and MSD. He has also received travel grants from BMS, Boehringer Ingelheim, Gilead, MSD and Janssen. The institution of HF has received unrestrictive grant support from ViiV, Gilead, Abbott, Janssen, Roche, BMS, MSD and Boehringer Ingelheim. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

AM and RK conceived the study, performed the analysis and wrote the first draft of the manuscript. TT contributed to R package documentation and programming. MS, NKC, WLY, JB, SY, TK, VA, HF, AC, MB, MC, EB, PS, KJM and HFG contributed to data collection and manuscript preparation.

Appendix: The Swiss HIV Cohort Study group

The members of the Swiss HIV Cohort Study group are: V. Aubert, M. Battegay, E. Bernasconi, J. Böni, H. C. Bucher, C. Burton-Jeangros, A. Calmy, M. Cavassini, G. Dollenmaier, M. Egger, L. Elzi, J. Fehr, J. Fellay, H. Furrer (Chairman of the Clinical and Laboratory Committee), C. A. Fux, M. Gorgievski, H. Günthard (President of the SHCS), D. Haerry (deputy of 'Positive Council'), B. Hasse, H. H. Hirsch, M. Hoffmann, I. Hösli, C. Kahlert, L. Kaiser, O. Keiser, T. Klimkait, R. Kouyos, H. Kovari, B. Ledergerber, G. Martinetti, B. Martinez de Tejada, K. Metzner, N. Müller, D. Nadal, D. Nicca, G. Pantaleo, A. Rauch (Chairman of the Scientific Board), S. Regenass, M. Rickenbach (Head of Data Center), C. Rudin (Chairman of the Mother & Child Substudy), F. Schöni-Affolter, P. Schmid, J. Schüpbach, R. Speck, P. Tarr, A. Trkola, P. Vernazza, R. Weber and S. Yerly.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting Information S1. Additional information, methods and sensitivity analysis.

Supporting Information S2. svisits:finding HIV transmission and serosorting pairs using shared clinic visit dates.

CHAPTER IV

"The cumulative impact of harm reduction on the Swiss HIV epidemic: cohort study, mathematical model and phylogenetic analysis"

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Description of personal contribution

The project was conceptualized by AM and RDK. AM performed the historical research and references collection, constructed the mathematical model and performed model fitting to data. AM extracted the data from the SHCS patient and resistance databases, performed quality control, constructed the phylogenetic trees, and analyzed the data using various statistical tools. AM produced all the tables and all the figures. AM wrote the first manuscript draft and the final version.

Research in context

In this paper, we integrate more than thirty-five years of data from high-quality sources to assess the population-level effectiveness of prevention measures against one of the key drivers of the global HIV epidemic:

HIV transmission among injecting-drug-users (IDUs) is the leading mode of transmission in Eastern Europe and Central Asia(1) and is recently re-emerging in the United States in the context of the growing heroin epidemic driven by overprescription of opioid painkillers (2–4). In Russia, a staggering figure of 98,000 new HIV cases were reported in 2015 alone, with 54% attributed to injecting drug use (5). Despite the immense scope of the problem, in many countries harm reduction remains non-existent or even illegal and as a result, an increasing number of opioid-dependent people are denied evidence-based interventions (6).

Although the effectiveness of individual harm reduction interventions like needle and syringe exchange programs and opioid substitution therapy is well established, population-level estimates of the cumulative impact of harm reduction are absent.

In this regard, Switzerland offers a unique experience. From the early eighties, Switzerland experienced one of the heaviest burdens of drug addiction (mainly Heroin and Cocaine) in Europe which manifested in the emergence of large open drug scenes and subsequently in an HIV outbreak. After adapting an extensive harm reduction package, transmission of HIV among IDUs in Switzerland almost ceased. To date, a quantitative evaluation of the cumulative impact of the implemented harm reduction measures has not been performed.

In this work, we combine a mathematical model with the data from the Swiss HIV Cohort Study (SHCS), the SHCS drug-resistance sequence database, national epidemiological data and data from previous works to demonstrate that overall, harm reduction prevented 15,903 HIV cases, 1980-2015. In addition, using a phylogenetic analysis, we demonstrated that the benefits of harm reduction extend beyond the population of injecting-drug-users, with 2,540 averted spillover infections from injecting-drug-users to the general population. These results highlight, based on the Swiss experience, the pivotal role of harm reduction, for the successful curbing of HIV transmission among IDUs and prevention of repercussions for the general population.

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MAJOR ARTICLE

The Cumulative Impact of Harm Reduction on the Swiss HIV Epidemic: Cohort Study, Mathematical Model, and Phylogenetic Analysis

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Background. Human immunodeficiency virus (HIV) transmission among injecting drug users (IDUs) is increasing in the United States due to the recent opioid epidemic and is the leading mode of transmission in Eastern Europe.

Methods. To evaluate the overall impact of HIV harm reduction, we combined (1) data from the Swiss HIV Cohort Study and public sources with (2) a mathematical model expressed as a system of ordinary differential equations. The model reconstructs the national epidemic from the first case in 1980 until 2015. Phylogenetic cluster analysis of HIV-1 pol sequences was used to quantify the epidemic spillover from IDUs to the general population.

Results. Overall, harm reduction prevented 15 903 (range, 15 359–16 448) HIV infections among IDUs until the end of 2015, 5446 acquired immune deficiency syndrome (AIDS) deaths (range, 5142–5752), and a peak HIV prevalence of 50.7%. Introduction of harm reduction 2 years earlier could have halved the epidemic, preventing 3161 (range, 822–5499) HIV infections and 1468 (range, 609–2326) AIDS deaths. Suddenly discontinuing all harm reduction in 2005 would have resulted in outbreak re-emergence with 1351 (range, 779–1925) additional HIV cases. Without harm reduction, the estimated additional number of heterosexuals infected by HIV-positive IDUs is estimated to have been 2540 (range, 2453–2627), which is equivalent to the total national reported incidence among heterosexuals in the period of 2007 to 2015.

Conclusions. Our results suggest that a paramount, population-level impact occurred because of the harm reduction package, beyond factors that can be explained by a reduction in risk behavior and a decrease in the number of drug users over time.

Keywords: harm reduction; HIV; injecting drug use; needle and syringe exchange; opioids.

Human immunodeficiency virus (HIV) transmission via injecting drug use remains one of the leading modes of transmission in Eastern Europe and many Asian countries (eg, China, Indonesia, Iran), and it is recently re-emerging in the United States as a result of the growing heroin epidemic, which is driven by overprescription of opioid analgesics [1–3].

Despite a large body of evidence on the effectiveness of harm reduction measures to halt the spread of HIV among people who inject drugs, there is still a large heterogeneity in the estimates [4]. These measures also remain politically controversial and are far from being universally implemented and accepted [5, 6]. As a result, the harm reduction coverage is still extremely low across the world and lags behind World Health Organization (WHO) targets [7].

From the early 1980s, Switzerland experienced one of the heaviest burdens of drug addiction (mainly heroin and cocaine) in Europe, which manifested in the emergence of large open drug scenes such as the "Platzspitz" ("Needle-Park") in Zürich. This resulted in an outbreak of HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) infections in this risk group, and Switzerland had the highest acquired immune deficiency syndrome (AIDS) incidence rate in Europe in 1988 [8]. After a growing public outcry, and considering the failure of repressive measures as the main response tool, a new progressive drug policy was gradually implemented that was based on "four pillars": prevention, therapy, harm reduction, and law enforcement [9].

The main harm reduction measures included the following: (1) extensive needle-exchange programs, ie, on-site distribution at open drug scenes, pharmacies, and syringe vending machines; (2) supervised drug consumption rooms; (3) low-threshold methadone programs; and (4) since 1994, a

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supervised, injectable medicinal heroin program. In parallel, a wide-reaching "STOP AIDS" campaign was launched with a tailored message for drug users, which emphasized the HIV risk in needle sharing [10]. Furthermore, HIV-infected current and former injecting drug users (IDUs) had broad access to antiretroviral drug treatment programs [11, 12].

From the public health perspective and particularly regarding HIV transmission, those efforts proved to be a phenomenal success. Despite a relatively low cessation rate of drug use and despite the fact that the prevalence of heroin addiction remained relatively stable [13], the transmission of HIV among IDUs in Switzerland dropped from a peak of 937 new cases in 1989 to a low of 2% (9 of 519) of all new infections in 2014, hence almost eliminating HIV transmission among IDUs.

To date, a quantitative evaluation of the cumulative impact of the implemented harm reduction measures has not been performed. In this study, we combine a mathematical model with the data from the Swiss HIV Cohort Study (SHCS), the SHCS drug-resistance sequence database, national epidemiological data, and data from previous works to perform the following: (1) estimate the counterfactual HIV incidence and prevalence among IDUs in absence, or with delayed introduction, of the harm reduction measures; (2) examine the effect of discontinuing harm reduction measures when the HIV epidemic among IDUs appears as under control; and (3) estimate the cumulative effect of the implemented harm reduction measures on the spillover of the epidemic to the general population in Switzerland.

METHODS

Ethics

We obtained ethical approval from the SHCS and written informed consent from all participants.

Swiss HIV Cohort Study and the Drug Resistance Database

The SHCS is an ongoing prospective cohort of HIV-positive individuals. The study prospectively enrolled patients since 1988, and some data were retrospectively ascertained until 1981. During the biannual outpatient visits, comprehensive clinical and behavioral data are collected [14]. In addition, for more than 60% of the participants, partial *pol* sequences are available. The representativeness of the SHCS was estimated to be high, with good coverage of marginalized and hard-to-reach populations, and is particularly good for subtype B, which is the predominant subtype in Switzerland [15].

Mathematical Model

We constructed a compartmental, deterministic transmission model represented as a nonlinear system of 32 ordinary differential equations (Figure 1). The model reconstructs the epidemic from the first introduced HIV case into the IDUs population in 1980 and is numerically solved until 2015. The modeled population corresponds to all heroin users in Switzerland. The model is divided into 3 meta-strata that represent a typical progression of an addiction course: (1) "non-injectors" represent people who smoke or snort heroin; (2) "active injectors" represent populations at risk of infection with HIV by sharing injection paraphernalia; and (3) "past-injectors" represent people covered by harm reduction that are still addicted to opioids, but do not contribute to the infectious pool anymore, since they switched to snorting/smoking or are in a methadone or supervised heroin program and permanently ceased injecting in a setting that facilitates transmission. The active injectors and the past-injectors are stratified into HIV susceptible and infected. All infected IDUs start in the undiagnosed compartment and can be diagnosed either in recent, chronic, or AIDS stage, with different rates. Since 1996, diagnosed individuals can transit to a combined antiretroviral treatment (cART) treated stage, with rates that depend on the disease stage and are increasing with calendar year to reflect transition to immediate treatment. Those rates were estimated from the SHCS based on CD4 counts as a proxy for disease stage (Supplementary Table 3). Except for past-injectors, which are covered by harm reduction by definition, each compartment is mirrored by a parallel harm reduction-covered strata to which individuals transit with an average rate that represents the harm reduction recruitment rate. Because the different harm reduction layers were overlapping in time (see Supplementary Figure 1), we do not model the separate effect of each measure (methadone, needle exchange, supervised injectable heroin, etc), but we use a harm reduction "package" [7] that was introduced in 1988, which means being covered by any of the harm reduction measures versus being missed by all of them. The exception to this pooled consideration of harm reduction is the restricted methadone program, because this was the main available measure before the introduction of the package, which allowed us to disentangle its effect. We assumed that IDUs covered by harm reduction had lower HIV transmission coefficient. This transmission rate, the harm reduction package recruitment rate, and other model parameters were determined by fitting the model using negative log-likelihood-distributed error to the annual number of new HIV cases and AIDS deaths in IDUs that were reported to the Swiss Federal Office of Public Health. See the Supplementary Data for a detailed description of the model, parametrization, and sensitivity analysis.

Phylogenetic Analysis

A large maximum likelihood phylogenetic tree with 19 604 Swiss sequences and 90 994 non-Swiss background sequences was constructed as previously described [16]. Introduction events into the general (heterosexual) population that originated from IDUs were detected by extracting all clusters that comprised only Swiss sequences and had at least 1 IDU and 1 heterosexual individual. For each IDU, the tree nodes were traversed back until the cluster either contained another IDU individual or a risk group that is other than an IDU or heterosexual, then the

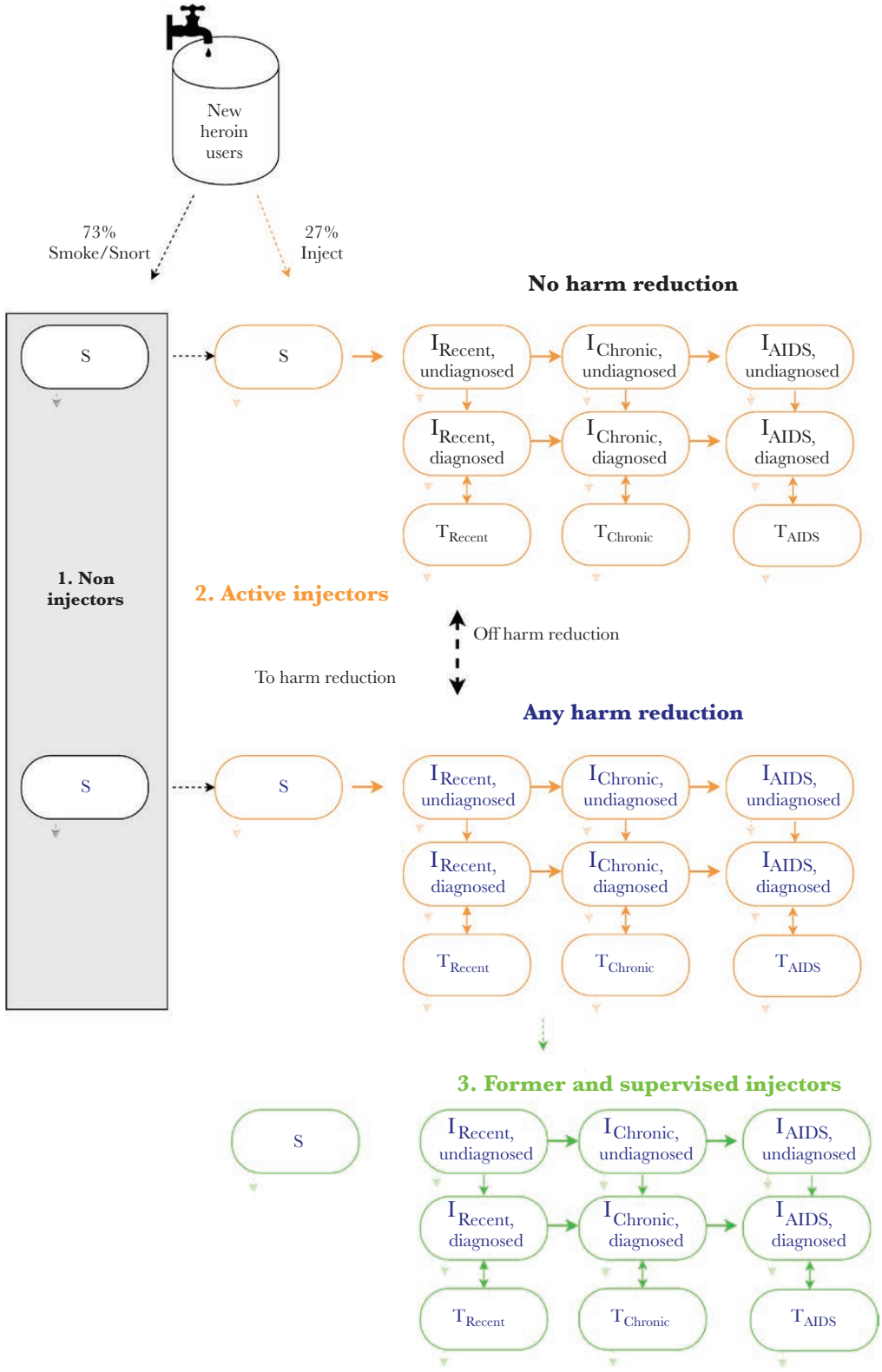

Figure 1. Graphical representation of the mathematical model. I, infected; S, susceptible; T, treated with cART (since 1996).

largest previous cluster was returned. This way, our analysis estimated not only the spillover population but also the further transmission of HIV within the heterosexual population caused by that spillover population. See the Supplementary Data for a detailed description of cluster analysis and the spillover calculation.

Analysis Tools

Statistical analysis was performed with R (version 3.2.3). The system of equations was solved using the package deSolve (version 1.14); the package "ape" (version 4.1) was used for phylogenetic analysis.

RESULTS

Injecting Drug Users in the Swiss HIV Cohort Study

Between 1983 and 2016, 4806 IDUs were enrolled in the SHCS, 3311 of those were most likely infected with HIV through sharing infected paraphernalia, and the remaining 1495 might have been infected via sharing or via sexual route.

The number of newly enrolled IDUs decreased with time from 553 newly registered in 1990, to 17 in 2016 (P for trend <.0001; Figure 2), hence accurately reflecting the drop of HIV incidence among IDUs in Switzerland [17]. Most IDUs were men (65.7%, 3157 of 4806; Supplementary Table 1) and were without university education (99.1%, 4761 of 4806). Both time to cART and subsequently the fraction of IDUs with AIDS-defining illness have decreased with time (P for trend .048), with almost immediate treatment initiation in 2010–2016, and 15.9% (11 of 69) of IDUs with AIDS-defining illness, compared with 48.0% (1441 of 2999) for IDUs that were diagnosed until 1990 (Fisher's exact test, $P < .0001$).

The period prevalence of HBV and HCV coinfections was high, with 78.3% (2312 of 2954; 1862 not tested) and 94.6% (2728 of 2883; 1923 not tested), respectively, for the entire period, and 58.2% (39 of 67; 2 not tested) and 73.1% (49 of 67; 2 not tested) in the last 7 years. This high prevalence of HBV and HCV alludes to a high fraction of nonassortative needle sharing, as expected in an open drug scene and assumed in our model.

Model Performance

The proposed model exhibits a qualitatively good fit, both to the annual number of newly diagnosed HIV cases among IDUs in Switzerland and to the annual number of reported AIDS deaths (Figure 3a and b, respectively). The model also catches, for the most part, the assumed dynamic of the population of problematic heroin users in Switzerland, with a peak during early 1990s and a subsequent gradual decline (Figure 3c). Finally, the model predicts HIV prevalence among IDUs in Switzerland, which falls in line with published estimates of approximately 10% between 1993 to 2000 [18]. Human immunodeficiency virus prevalence and the number of heroin users were deliberately not used for model fitting, to serve as an additional quality check for the prudence of our model; nevertheless, the dynamics of those compartments is captured well by the model.

The Combined Effect of Harm Reduction Measures, No Harm Reduction, and Sudden Discontinuation

First, we examined the extreme—yet relevant to other countries—worst-case scenario of no harm reduction at all since 1980, which required transferring the individuals on restricted methadone—that was available since late 1970s—to model compartments not covered by any harm reduction, from the start of the simulation. This resulted in 15 903 (range, 15 359–16 448) additional infections until the end of 2015 (Figure 4a and Figure 5), 5446 new additional AIDS deaths (range, 5142–5752) (Figure 4b), and a peak HIV prevalence of 50.7% (Figure 4c). Next, we examined whether a sudden discontinuation of all harm reduction services, after several years of low incidence, will result in a renewed outbreak. Our model shows that suddenly discontinuing all harm reduction in the year 2000

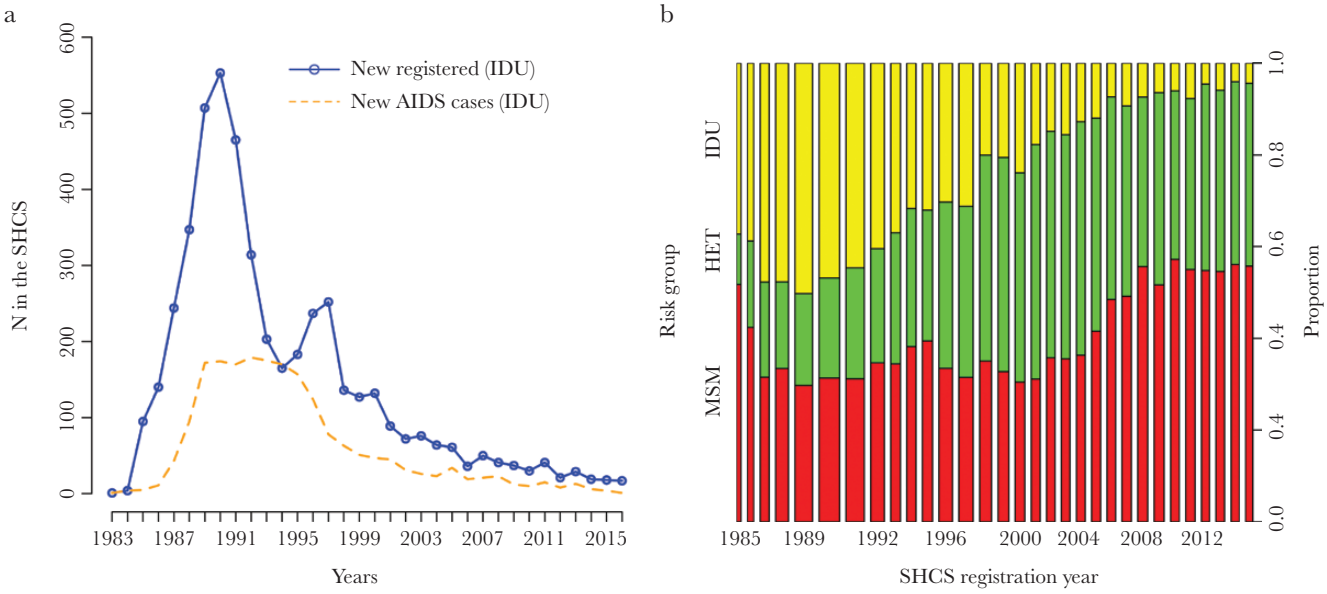

Figure 2. (a) New registered injecting drug users (IDUs) and new acquired immune deficiency syndrome (AIDS) cases in the Swiss HIV Cohort Study (SHCS). (b) The fraction of IDUs (yellow) out of all newly registered patients in the Swiss HIV Cohort Study, by registration year.

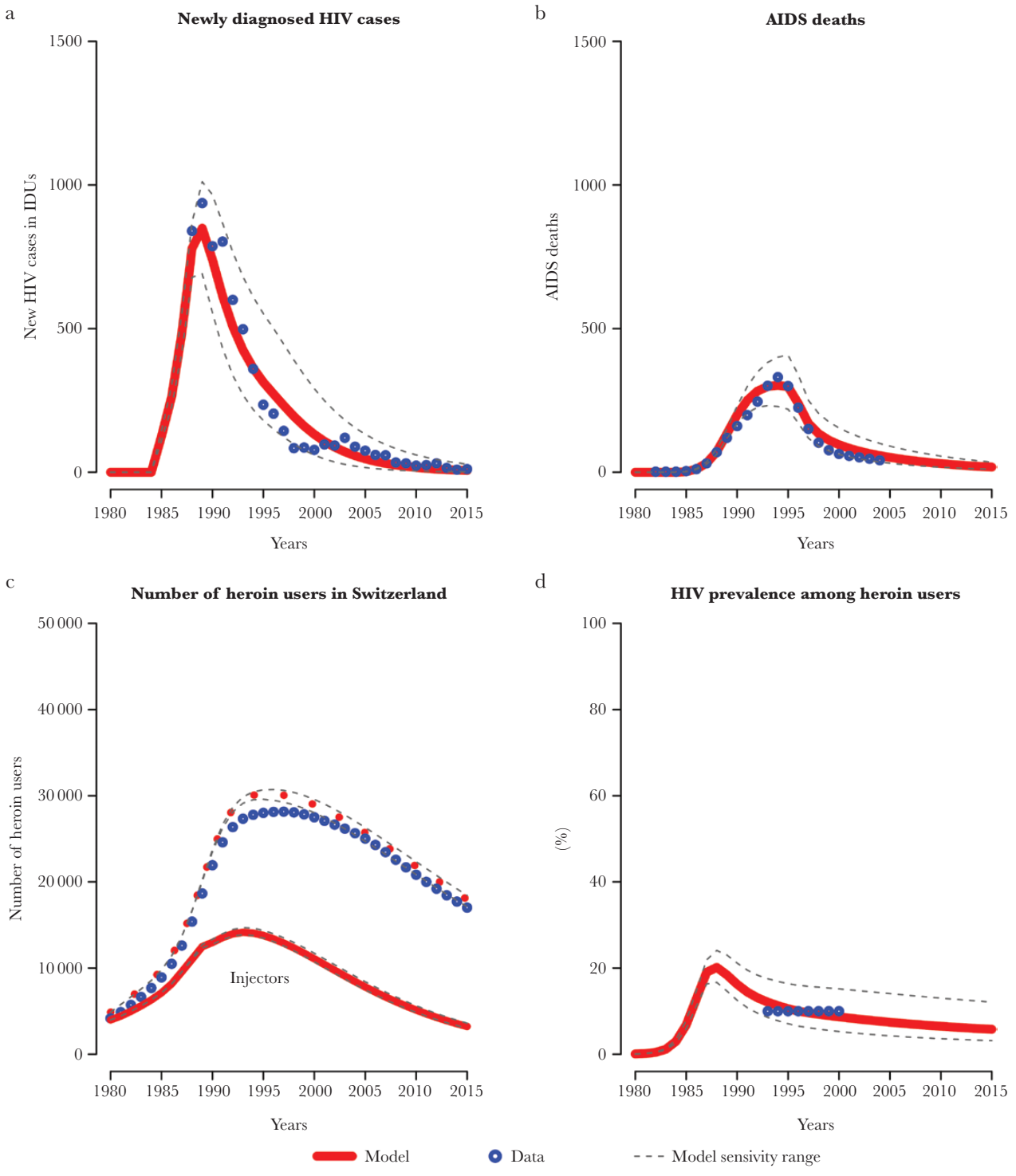

Figure 3. Model fit versus reported data. Source: (a) incidence [17, 20, 37], (b) acquired immune deficiency syndrome (AIDS) deaths [17], (c) number of heroin users [13] (extrapolated 2010–2015), (d) human immunodeficiency virus (HIV) prevalence [18].

(10 years after the incidence peak) or 2005 would have resulted in outbreak re-emergence up to 2015 in both scenarios, with 4965 (range, 3420–6511) and 1351 (range, 779–1925) additional HIV cases, respectively (Figure 4a and Figure 5). However, the re-emergence rate is twice as slow when harm reduction is discontinued in 2005 compared with 2000, with linear slopes of 20.2 (95% confidence interval [CI], 18.8–21.6) and 40.5 (95% CI, 39.6–41.3) new cases, respectively, for the first 5 years after discontinuation. Because of immediate cART in the recent years, discontinuation in 2005 would have had limited to no

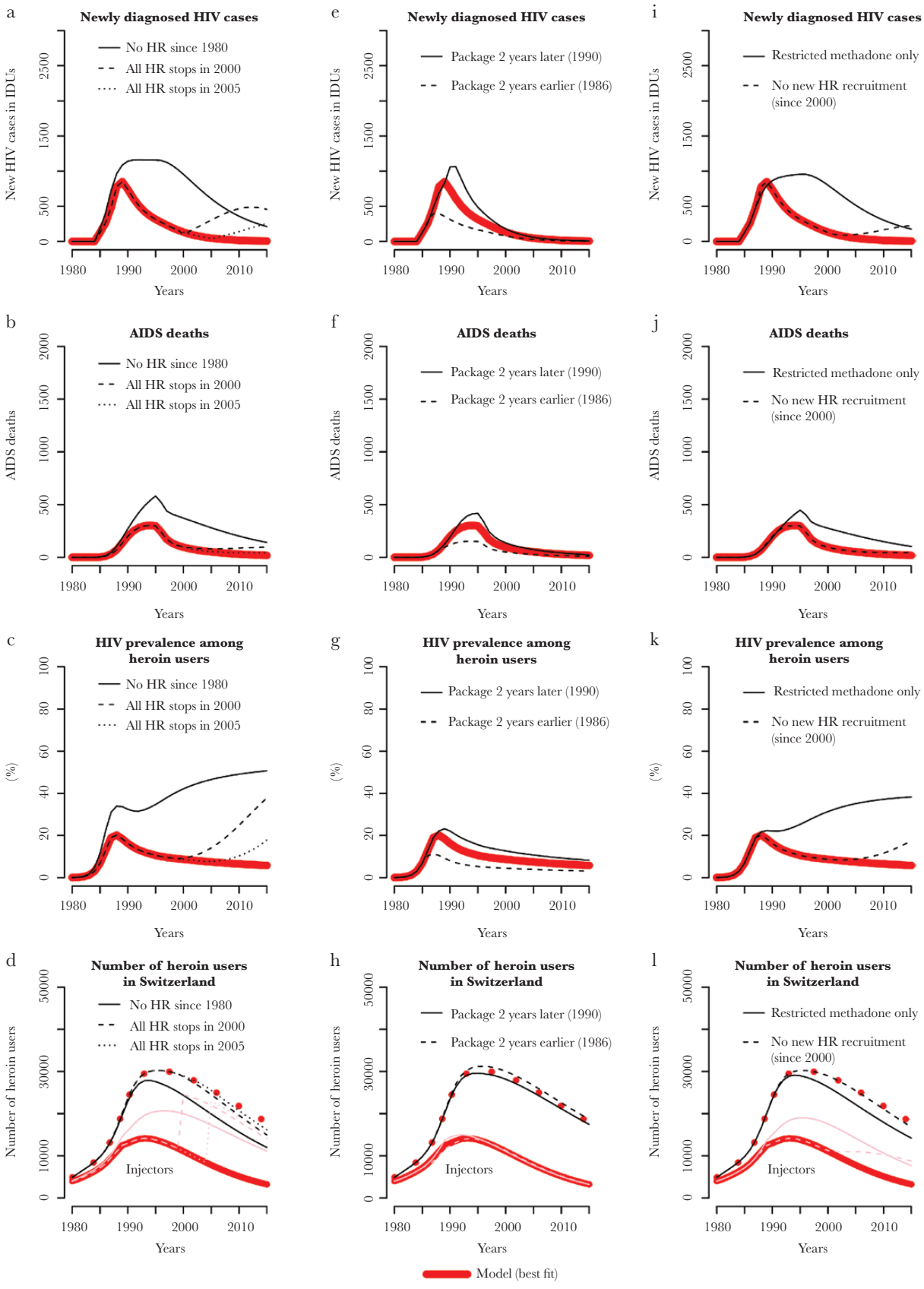

Figure 4. Seven counterfactual scenarios examined in this study. (Column 1, a–d) No harm reduction (HR) since 1980, All HR stops in 2000, All HR stops in 2005. (Column 2, e–h) HR package introduced 2 years later (1990), HR package introduced 2 years earlier (1986). (Column 3, i–l) Restricted Methadone only (entire period 1980–2015), no new HR recruitment since 2000.

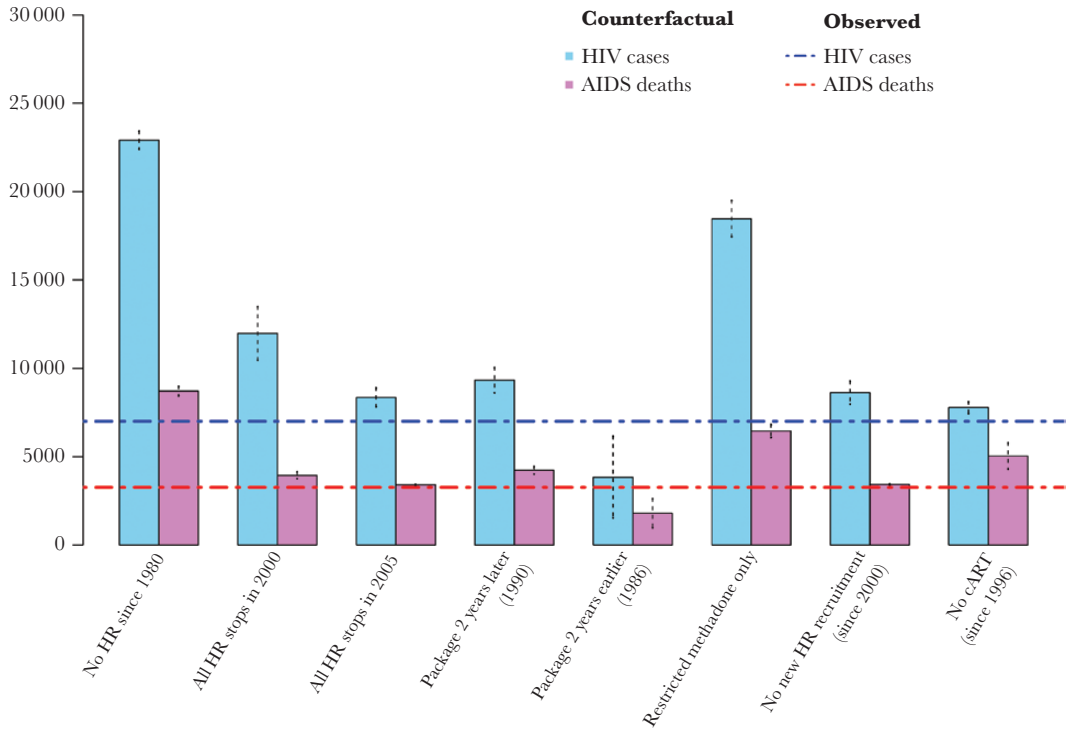

Figure 5. Total number of human immunodeficiency virus (HIV) cases and acquired immune deficiency syndrome (AIDS) deaths among injecting-drug-users in Switzerland, 1980–2015, across the examined scenarios and compared with the reported data (as model best fit). The no-combined antiretroviral treatment (cART) scenario is shown as well. Black dashed bars represent sensitivity analysis range. HR, harm reduction.

effect on the number of AIDS deaths with 153 (range, 101–206) additional deaths (Figure 4b).

Delayed or Earlier Introduction

A delay of 2 years in the introduction of the harm reduction package, ie, 1990 instead of 1988, would have resulted in 2325 (range, 1580–3071) additional HIV cases (Figure 4e and Figure 5) and 971 additional AIDS deaths (range, 724–1219) (Figure 4f). On the other hand, a 2 years earlier introduction (ie, 1986) could have halved the epidemic, preventing 3161 (range, 822–5499) HIV infections and 1468 (609–2326) AIDS deaths, with a peak HIV prevalence among IDUs that would have never exceeded 11.3 % (range, 8.5%–15.3%).

Only Restricted Methadone and No New Recruitment Since 2000

Next, we examined the effect of not introducing the extensive harm reduction package but continuing with high-threshold methadone only, with the same recruitment rate as before 1988 (~8.5% per year; Supplementary Data). This would have resulted in 11 462 additional HIV cases (range, 10 399–12 526) (Figure 4i and Figure 5) and 3190 (range, 2793–3588) additional AIDS deaths (Figure 4j). It is notable that restricted methadone is still superior to the scenario with no harm reduction at all, with 4441 prevented cases and 2256 fewer deaths. Finally, we explored a less radical discontinuation scenario, in which individuals that are covered by harm reduction remain in the covered compartment (with the same dropout rate); however, since 2000, there is no new recruitment to the harm reduction covered compartments. This scenario emulates a harm reduction budget cut plot. This would have resulted in a slow re-emergence with 1616 additional HIV cases (range, 938–2295) (Figure 4i) with no substantial increase in additional AIDS deaths (range, 114–235) (Figure 4j).

The Effect of Combined Antiretroviral Treatment

Although, chronologically, the epidemic reached its peak and began to decline before cART introduction in 1996, we still observe a moderate protective effect of cART (the harm reduction-related parameters were not changed in this scenario), with 771 (range, 401–1142) new HIV cases prevented by cART alone until the end of 2015, and—as expected—an ample effect on AIDS deaths, with 1771 (range, 991–2552) prevented deaths (Figure 5 and Supplementary Figure 3).

Spillover to the General Population

The phylogeny contained 4235 sequences from 2399 SHCS IDUs, with 94.3% (2262 of 2399) harboring subtype B. Cluster analysis showed 499 heterosexuals clustered with IDU in Swiss-only clusters, which were linked to 358 putative cross-risk-group introduction events (Supplementary Figure 4) in which the phylogenetically closest IDU was male in 60.3% (216 of 358) and female in 39.7% (142 of 358). In absence of any harm reduction (scenario a), the estimated additional number of heterosexuals whose infection originated from HIV-positive IDUs

is estimated to have been 2540 (range, 2453–2627) new infections, which is comparable to the total national HIV incidence among heterosexuals in the entire period from 2007 to 2015 (n = 2476, Federal Office of Public Health [19–21]).

DISCUSSION

According to UNAIDS, in Eastern Europe and Central Asia, 51% of all newly diagnosed HIV is attributed to people who inject drugs [22]. However, only 7% to 15% of all IDUs in Eastern Europe have access to needle and syringe programs, whereas for opioid substitution treatment the coverage is approximately 1% [3], and it remains illegal in Russia. Likewise, the Western-Europe, North-America, and Australasia region combined have not yet reached the WHO middle-coverage target of 20% for needle and syringe programs [7].

Our model estimates that a very high prevalence of HIV (~50%) among IDUs would have occurred in the absence of harm reduction. More importantly, our model takes into account both the overall decrease in heroin consumption as well as the decreasing number of injectors. Thus, the high prevalence in the absence of harm reduction is predicted to have occurred despite those general trends of drug use. This counterfactual estimate is also in line with historical seroprevalence data from socioeconomically comparable areas that had little to no harm reduction at that time. Frankfurt, Germany, had a large open drug scene, with HIV prevalence of 73.7% in 1994 [23], in Spain the prevalence was 63% in 1996 [24], and in northern Italy the prevalence was 49% in 1989 [25]. Some areas in the United States also exhibited high HIV prevalence, with 61% in New York [26] and 60% in New Haven, Connecticut [27], during the early 1990s. In Eastern Europe, and especially in Russia, which exercises a repressive approach toward IDUs and repulsion of the harm reduction concept on the political level, a 37% prevalence was estimated in 2003 [3]. In Estonia, the rate was as high as 72% [3].

Considering the low incidence of HIV among IDUs in Switzerland in the recent years, there is a growing debate on whether the funds invested in harm reduction can be safely allocated elsewhere. In 2016, the canton of Zürich decided to cut 4.5 million Swiss Francs from the drug-addiction treatment programs until 2019 [28]. Our study shows that suddenly stopping harm reduction measures, even several years after the epidemic appears as under control, can lead to a new outbreak. This result is supported by a recent experience from Greece, a country with a historically low HIV incidence among IDUs (1.5% to 4.5% of all new infections during 2000–2010) [29]. In 2011, due to the fiscal crisis and severe austerity, the harm reduction measures were underperforming [30]. Until the first 8 months of 2013, 1000 new HIV cases among IDUs have already been diagnosed [31]. After harm reduction—in form of needle and syringe exchange and opioid-substitution—was scaled up again, HIV incidence was reduced 5-fold within 1 year [32].

Our estimates show a moderate impact of cART on curbing HIV transmission among IDU in Switzerland. This can be attributed to 2 factors: (1) cART was introduced in 1996 after the epidemic was already contained by the harm reduction measures, which started in part in 1988; and (2) the effect of cART is partly undermined by lower adherence among IDUs [33], which was also reflected in our model. However, as expected, cART prevented a large number of AIDS deaths among IDUs.

Our model has several benefits and can be adjusted for the following factors: (1) the decrease in the number of injectors with time; (2) the possible reduction in risk behavior even in people who are not reached by any harm reduction due to overall awareness of HIV [34]; and (3) the decrease in needle sharing by IDUs who are aware of their HIV-seropositive status and are concerned of infecting others [35]. Because the extent of the relevance of those developments to the Swiss settings is uncertain, we speculate that we might have underestimated the effectiveness of the combined harm reduction measures and that our estimates lay on the conservative side. This is further supported by the fact that, due to scarcity of data, we could not account for cocaine-only injectors; however, injectors of cocaine and heroin ("Speedball") were accounted for in our model. In addition, our model has the advantage of being applicable to the current opioid analgesics-driven HIV epidemic, because it accounts for the transition from a noninjecting to injecting drug administration mode.

Our model is limited because it does not differentiate between the different measures implemented, except for restricted methadone. However, in this work, we were a priori interested in cumulative estimates. Our model also only accounts for sexual transmission within but not between the 3 meta-strata. Nonetheless, the contribution of sexual transmission is expected to be of secondary importance due to an 8-fold higher per-act transmission probability for needle sharing [36]. Finally, as it is often in modeling studies, the uncertainty ranges of our predictions might be underestimated.

Indeed, not all countries affected by an HIV epidemic among IDUs possess the resources that were available in Switzerland. However, the unit costs of harm reduction interventions are relatively low and are estimated to be highly cost effective [7] and, in light of the results presented here, might even be cost saving. In addition, we demonstrated that the benefits of harm reduction extend beyond the population of IDUs, with thousands of averted spillover heterosexual infections. Similar studies are needed for the HCV epidemic, which affects this population even more severely than HIV.

CONCLUSIONS

In summary, our results highlight, based on the Swiss experience, the pivotal role of harm reduction for successful curbing of HIV transmission among IDUs and prevention of grave repercussions for the general population.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Members of the Swiss HIV Cohort Study. Anagnostopoulos A., Battegay M., Bernasconi E., Böni J., Braun D. L., Bucher H. C., Calmy A., Cavassini M., Ciuffi A., Dollenmaier G., Egger M., Elzi L., Fehr J., Fellay J., Furrer H. (Chairman of the Clinical and Laboratory Committee), Fux C. A., Günthard H. F. (President of the SHCS), Haerry D. (Deputy of "Positive Council"), Hasse B., Hirsch H .H., Hoffmann M., Hösli I., Huber M., Kahlert C., Kaiser L., Keiser O., Klimkait T., Kouyos R. D., Kovari H., Ledergerber B., Martinetti G., Martinez de Tejada B., Marzolini C., Metzner K. J., Müller N., Nicca D., Paioni P., Pantaleo G., Perreau M., Rauch A. (Chairman of the Scientific Board), Rudin C. (Chairman of the Mother & Child Substudy), Scherrer A. U. (Head of Data Centre), Schmid P., Speck R., Stöckle M., Tarr P., Trkola A., Vernazza P., Wandeler G., Weber R., Yerly S.

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CHAPTER V

"High rates of subsequent asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection"

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Description of personal contribution

The project was conceptualized by HFG and DB. AM performed several rounds of quality control and performed univariable and multivariable statistical analysis, using various approaches (Mixed-model logistic regression and Cox-regression). AM produced all the tables and the figures and wrote the results and the statistical methods sections. DB and AM wrote the first draft. AM performed additional analysis for the revision.

Research in context

One of the most pertinent issues concerning the HIV positive population is the dramatic increase in coinfections with sexually transmitted infections (STIs), in particular in Men-who-have-Sex-with-Men (MSM) and hence there is high urgency for studies that help to optimize screening and prevention strategies.

In this work, we systematically describe the characteristics and risk factors of individuals with a sexually transmitted infection (STI) in a well-characterized cohort of mainly MSM with a prior diagnosis of primary HIV-1 infection. We prospectively collected the data from 214 individuals enrolled in both the Zurich Primary HIV Infection Study (ZPHI) and the Swiss HIV Cohort Study (SHCS).

The prospective and longitudinal study design allowed us to calculate prevalence and incidence rates of most common STIs (Gonorrhoea, Chlamydia, Syphilis etc.) and to test a variety of risk factors against having a STI. In our unique patient population, we found a very high STI prevalence (33%), mostly presenting asymptomatic, and identified three independent factors associated with a positive STI screen: i) anal intercourse, ii) reporting condomless sex, and iii) reporting any recent drug use. These results highlight the importance of a more frequent (3-monthly) screening in high-risk populations as identified in this study, and demonstrate the potential of relying on self-reported sexual risk behaviour and drug use data for screening prioritization

Clinical Infectious Diseases

MAJOR ARTICLE

High Rates of Subsequent Asymptomatic Sexually Transmitted Infections and Risky Sexual Behavior in Patients Initially Presenting With Primary Human Immunodeficiency Virus-1 Infection

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Background. Knowledge of the risk factors of individuals with an asymptomatic sexually transmitted infection (STI) is essential for implementation of targeted STI screening strategies.

Methods. Between June 2015 and January 2017, an STI screening was offered to all participants in the Zurich Primary human immunodeficiency virus (HIV)-1 Infection study. Patients were tested for gonorrhea, chlamydia, syphilis, and hepatitis C virus (HCV).

Results. Of 214 participants, 174 (81%) were screened at least once. Most patients were men who have sex with men (MSM) (87.4%). Presenting with a primary HIV infection was associated with higher odds for later risky sexual behavior, as compared with presenting in the chronic phase (odds ratio [OR], 5.58; 95% confidence interval [CI], 3.68–8.8). In total, 79 STIs were detected, reflecting a high period prevalence of 33.3% (58 of 174 patients). Sixty-six percent of patients (52 of 79) were asymptomatic. Most common STIs were chlamydia (50.6%; 40 of 79 patients), gonorrhea (25.3%; 20 of 79), and syphilis (19%; 15 of 79). In a multivariable model, engaging in insertive (OR, 6.48; 95% CI, 1.14–36.76) or both insertive and receptive (4.61; 1.01–20.96) anal intercourse, STI symptoms (3.4; 1.68–6.89), and condomless sex (2.06; 1.14–3.74) were positively correlated with a positive screening result. The hazard of an incident STI increased with the presence of STI symptoms (hazard ratio, 3.03; 95% CI, 1.17–7.84) and any recent drug use (2.63; 1–6.9).

Conclusions. A trimonthly STI screening including asymptomatic individuals should be considered in this population, particularly in MSM who report sexual risk behavior.

Keywords. primary HIV-1 infection; sexually transmitted infection; sexually transmitted disease; asymptomatic; screening.

Rates of sexually transmitted infections (STIs) are rising in men who have sex with men (MSM) and individuals with human immunodeficiency virus (HIV) infection [1–5]. Patients with a primary HIV infection treated with early antiretroviral therapy (ART) represent a highly sexually active population and contribute substantially to this epidemic [6, 7]. Recognizing the high prevalence of STIs in the HIV-infected and HIV-uninfected MSM population, the Centers for Disease Control and Prevention recommends routine laboratory screening for common STIs (ie, syphilis, gonorrhea, chlamydia) at least annually for sexually active MSM and a more frequent STI screening (ie, at 3–6-month intervals) for MSM who have multiple or anonymous partners [2].

During recent years it has been recognized that STIs commonly present as asymptomatic, predominantly affect MSM, and may occur in multiple anatomic sites [4, 8–12]. An intensive 3-site screening (ie, rectal, pharyngeal, urethral) is therefore needed to detect asymptomatic STIs [13] appropriately. The rationale to direct efforts into diagnosing and treating asymptomatic STIs include (1) the identification of transmitter pools and the reduction of onward transmission, (2) the prevention of disseminated infection, (3) the identification of other STIs through partner notification, and (4) the prevention of the emergence of resistance as the oropharyngeal niche provides an enabling environment for horizontal transfer of genetic material from commensal *Neisseria* sp. and other bacterial species to *Neisseria gonorrhoeae* (20) [14]. A recently published mathematical model [15] showed that the introduction of a routine STI screening every 6 months could reduce the incidence of *Chlamydia trachomatis* and HIV infection by 15% and 4%, respectively. However, 3-site

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screening at 3–6-month intervals is both time and cost consuming [16].

Given the high burden of STIs, especially in HIV-infected MSM, and the challenges to identify them accurately, better knowledge of the characteristics and risk factors of patients with STIs, specifically asymptomatic STIs, is crucial to better guiding healthcare providers on whom to screen. In this regard, data are particularly scarce concerning HIV-infected individuals whose initial diagnosis was of acute or recent infection. Understanding the risk profile and behavioral attitudes associated with STI acquisition could help identify individuals in need of regular and intensified STI screening. Here we report on the characteristics of 214 HIV-infected predominantly MSM enrolled in the Zurich Primary HIV-Infection (ZPHI) study and the risk factors associated with STI detection in this population.

METHODS

Study Population

All patients had a documented primary HIV infection and are enrolled in the ZPHI, an open-label, nonrandomized, observational, single-center study (ClinicalTrials.gov, ID NCT00537966) [17]. The ethics committee of the Canton of Zurich approved the study protocol, and written informed consent was obtained from all patients. Acute or recent primary HIV infection was confirmed in all patients, as published elsewhere [17–19]. Acute HIV infection was defined as acute retroviral syndrome (ARS), negative or indeterminate HIV line immunoassay results in the presence of positive p24-antigen, and/or detectable HIV-1 RNA, or as a documented seroconversion with or without symptoms during the past 90 days. Recent infection was defined as possible ARS, positive HIV Line Immunoassay results, detectable HIV-RNA, and a negative HIV glycoprotein 120 avidity or detuned assay result, or as a documented acute HIV-1 infection but with referral to our center >90 but <180 days after the presumed date of infection.

All patients considered in this study are also participants in the Swiss HIV Cohort Study (SHCS) (www.shcs.ch) [20]. The SHCS is a prospective cohort with ongoing enrollment for HIV-infected individuals in Switzerland that started in 1988. Clinical, laboratory, and sociodemographic information is collected every 6 months, including information about sexual behavior. In addition, detailed treatment history is kept for all patients.

Data Collection

Between 1 June 2015 and 31 January 2017 we offered regular STI screenings to all patients from the ZPHI study who had a clinical visit. Patients had to be screened at least once to be included in the analysis. They were then also asked to complete a behavioral questionnaire that assessed the sexual behavior and symptoms related to a possible STI in the preceding 3 months (Supplementary Material). From June 2015 to June 2016, we performed separate polymerase chain reaction (PCR) tests for each anatomic site to determine the sites of infection. To reduce costs, the sampling procedure was simplified to a pooled STI screening from July 2016 to January 2017.

Laboratory Tests

Swabs obtained from the urethra, rectum and pharynx were tested by PCR for *C. trachomatis* and *N. gonorrhoeae*. Blood was tested for syphilis by Treponema-pallidum-Particle-agglutination (TPPA), and VDRL and/or rapid plasma reagin tests were done in the event of a positive TPPA result. Patients were also tested yearly for anti–hepatitis C virus (HCV) immunoglobulin G. In patients presenting with elevated transaminase levels, with >50 U/L as the upper limit of a normal value, blood samples were also tested for hepatitis C by PCR. For detailed testing information, see Supplemetary box 1. For specific treatment see Supplemetary box 2.

Statistical Analysis

Statistical analysis was performed using R software, version 3.3.2. Bivariate *P* values were calculated using the Fisher exact test for categorical variables and the Wilcoxon test for continuous variables. Bivariate *P* values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. For multivariable analysis, with the outcome of STI diagnosis during any of the screening visits, a generalized linear mixed model logistic regression was used with a per-patient random intercept. Apart from age at diagnosis and CD4 cell count, which were included a priori, variables that were significant in the bivariate analysis (Benjamini-Hochberg $P < .05$) were included in the multivariable model, if not strongly collinear with another independent variable. Square root transformation was performed on the CD4 cell counts to fulfill model assumptions [21]. The Cox proportional hazard model was used for the prospective subsample with a negative baseline, with the same selection strategy for variables.

RESULTS

Visits, Patients, and Period Prevalence of STI

During the study period, 623 follow-up visits from 214 patients occurred with a median of 3 visits per patient (range, 1–4 visits); 174 of 214 patients (81%) agreed to be screened at least once, accounting for 334 visits (Figure 1) with a valid STI screening. In total, 79 STIs were detected in 58 patients during 71 visits, reflecting a high period prevalence of 33.3% (58 of 174; 95% confidence interval [CI], 26.4%-40.9%). Even if it is assumed that all 40 untested patients had no STIs, this would still imply a high period prevalence of 27.1% (58 of 214; 21.3%-33.6%). In 8 visits, >1 STI was detected in the same patient. The most prevalent reported reason for the 40 patients' study nonparticipation was a reported belief that their risky behavior was insufficient to warrant screening (Figure 1).

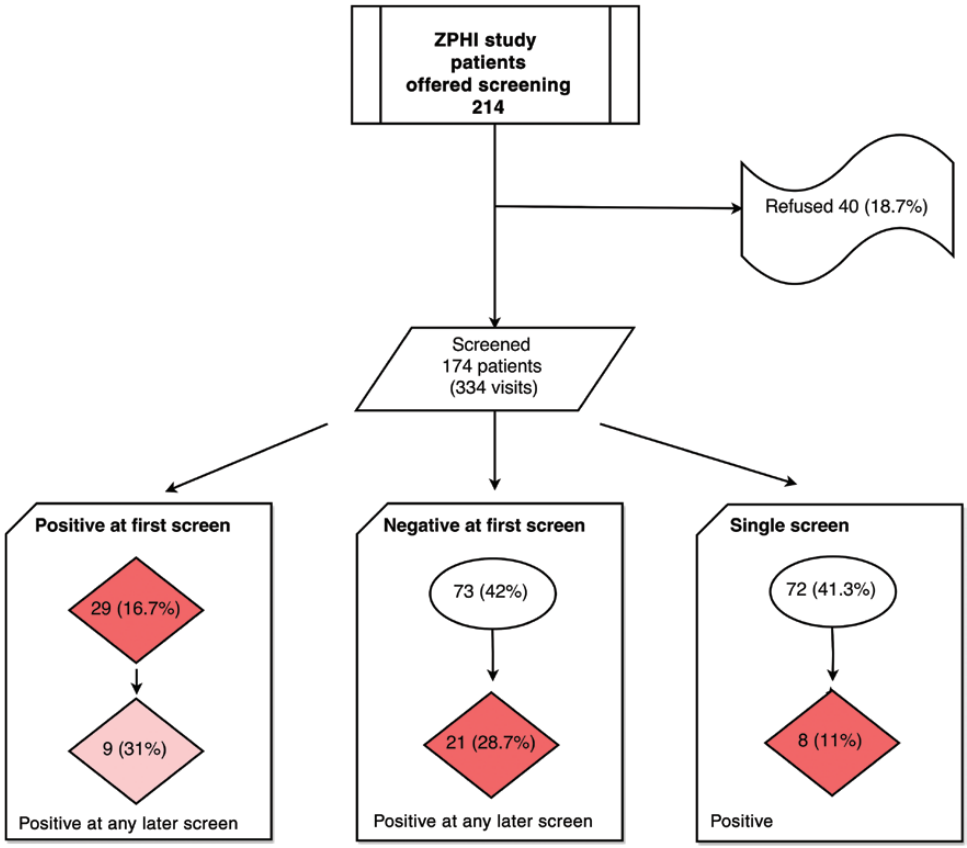

Figure 1. Flow chart of the study. Abbreviation: ZPHI, Zurich Primary HIV-Infection.

The most common STIs were chlamydia (50.6%; 40 of 79), gonorrhea (25.3%; 20 of 79), and syphilis (19%; 15 of 79) (Table 1). The stage of syphilis infection included stage 1 (n = 7; 47%), stage 2 (n = 3; 20%), early latent (n = 4; 27%), and neurosyphilis (n = 1; 6%). A large fraction (of the detected STIs 65.8%; 52 of 79) were asymptomatic. For both chlamydia and gonorrhea, the rectum was the most prevalent anatomic site of infection, accounting for 62.5% (20 of 32; 8 unknown owing to pooled testing) and 53.3% (8 of 15; 5 unknown owing to pooled testing), respectively (Supplementary Table S1). The 3 detected HCV infections were considered incident infections because all 3 individuals had a prior negative HCV antibody test recorded in the SHCS database, presented with recently elevated liver transaminase levels, and reported risky sexual behavior and/or drug use in the preceding months.

Table 1. Sexually Transmitted Infections (STIs) Detected Stratified By Presence of STI Symptoms

	STIs Detected, No. (%)		
Type of STI	Total	Symptomatic	Asymptomatic
Chlamydia	40 (50.6)	11 (27.5)	29 (72.5)
Gonorrhea	20 (25.3)	6 (30)	14 (70)
Syphilis	15 (19)	9 (60)	6 (40)
Acute hepatitis C	3 (3.8)	0 (0)	3 (100)
HSV infection[a]	1 (1.3)	1 (100)	0 (0)
Total	**79 (100)**	**27 (34.2)**	**52 (65.8)**

Abbreviations: HSV, herpes simplex virus; STI, sexually transmitted infection.

[a]Accidental finding; HSV was not screened for systematically.

HIV Diagnosis During Primary Infection and Subsequent Risky Sexual Behavior

First, we examined whether presentation with a documented primary HIV infection was associated with a higher rate of later risky sexual behavior, compared with HIV diagnosis during the chronic phase. We compared 169 MSM ZPHI patients who initially presented with documented primary HIV infection and were offered STI screening in this study with 5105 MSM from the SHCS without a documented primary HIV infection. The outcome variable was reported condomless sex with an occasional partner at least once after diagnosis of HIV infection. After adjustment for age at diagnosis, year of diagnosis, and first CD4 cell count, diagnosis of a primary HIV infection was associated with a 5-fold higher odds for later risky sexual behavior, compared with diagnosis during the chronic phase (adjusted odds ratio [OR], 5.58; 95% CI, 3.68–8.8) (Table 2).

Factors Correlating With STI Detection

Only 1 STI was detected in the heterosexual risk group, with the remaining 98.6% (70 of 71) of visits with an STI belonging

Table 2. Standard Logistic Regression for Correlation Between Presentation With Primary Human Immunodeficiency Virus (HIV) Infection and Risky Sexual Behavior at Any Time Point After HIV Diagnosis[a]

	Univariable Model		Multivariable Model	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age at HIV diagnosis	0.99 (.98–.99)	<.001	0.98 (.98–.99)	<.001
Year of HIV diagnosis	1.04 (1.04–1.05)	<.001	1.04 (1.04–1.05)	<.001
First CD4 cell count, cells/μL				
<200 (reference)	1	…	1	…
200–500	1.73 (1.5–1.99)	<.001	1.51 (1.3–1.75)	<.001
>500	1.82 (1.57–2.13)	<.001	1.6 (1.36–1.87)	<.001
HIV infection at presentation				
Chronic (reference)	1	…	1	…
Primary HIV	7.9 (5.25–12.41)	<.001	5.58 (3.68–8.8)	<.001

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

[a]In this analysis, 169 men who have sex with men (MSM) in the Zurich Primary HIV-Infection study, who initially presented with a primary HIV infection and were offered screening for sexually transmitted infection in this study were compared with 5105 MSM from the Swiss HIV Cohort study without a documented primary HIV infection. The outcome variable was defined as reporting condomless sex with occasional partner at least once after HIV diagnosis. All variables were included in the multivariable model.

to the MSM risk group. In a bivariate analysis, compared with visits without STI detection, detection of an STI during a visit was significantly associated with a more recent year of HIV diagnosis (median year, 2012 [interquartile range (IQR), 2009–2014] vs 2010 [2007–2013]; $P = .009$) and hence with a shorter time since HIV diagnosis (median, 3.2 vs 5.3 years; $P = .006$). STI detection was also correlated with having anal intercourse (96% [68 of 71] vs 80% [21 of 263]; $P = .03$), a higher number of sexual partners (median, 3 [IQR, 2–6.5) vs 2 [1–4]; $P < .001$), reports of condomless sex (59.2% [42 of 71] vs 33.8% [89 of 263]; $P = .001$) and reports of STI symptoms (33.8% [24 of 71] vs 9.1% [24 of 263]; $P < .001$) (Table 3).

In a multivariable model restricted to MSM, the number of years since HIV diagnosis (OR, 0.87; 95% CI, .8–.96) and √CD4 cell count (0.93; .87–.99) were negatively associated with the odds of having a positive STI test result (Figure 2A). On the other hand, engaging in insertive (OR, 6.48; 95% CI, 1.14–36.76) or both insertive and receptive (4.61; 1.01–20.96) anal intercourse in the last 3 months, reporting STI symptoms (3.4; 1.68–6.89,) and reporting condomless sex (2.06; 91.14–3.74) were all positively correlated with the odds for a positive screening result.

Incidence Rate and Survival Analysis

Seventy-three patients were screened more than once and had a first negative screening result, which served as a baseline for the incidence analysis. Overall, 21 patients had ≥1 incident STI during a total of 37.2 person-years, which corresponds to an incidence density rate of 56 (95% CI, 35–86.3) per 100 person-years. When restricted to MSM only (93.2%; 68 of 73), the incidence rate was 61.6 (95% CI, 38.2–94.2) per 100 person-years.

In a bivariate analysis, incident STI was associated with a higher number of sexual partners (median, 3 [IQR, 2–6] vs 2 [1–3.2l]; $P = .041$), reporting of STI symptoms (38.1% [8 of 21] vs 9.6% [5 of 52]; $P = .05$), and any drug use in the last 6 months (38.1% [8 of 21] vs 5.8% [3 of 52]; $P = .04$) (Supplementary Table S2). In a multivariable Cox proportional hazard model restricted to MSM only, the hazard of an incident STI increased with the presence of STI symptoms (hazard ratio, 3.03; 95% CI, 1.17–7.84; $P = .02$) and any recent drug use (2.63; 1–6.9: $P = .049$) (Figure 2B).

Finally, considering the above-mentioned finding that any recent drug use was associated with STI risk, we explored data about use of specific substances (Table 4). None of the examined MSM reported intravenous heroin or cocaine use. However, a strong bivariate association was found between incident STI and recent use of noninjectable cocaine, cannabis, and other noninjectable drugs (as a group), (adjusted $P = .005$, .01, and .006, respectively). More specifically, recent use of ecstasy or gamma-hydroxybutyric acid was significantly associated with an incident STI, with rates of 28.6% (6 of 21) versus 0 of 47 ($P = .003$), and 19% (4 of 21) versus 0 of 47 ($P = .01$), respectively.

DISCUSSION

In this prospective analysis of mainly Swiss MSM with a prior diagnosis of primary HIV infection treated with suppressive ART during the study, we found a very high period prevalence and incidence rate of STIs. In line with the literature, the majority of the detected STIs were asymptomatic, and the most affected anatomic site was the rectum [1, 4, 8, 9, 11, 13, 22–34]. We identified 3 independent factors predictive of a positive STI screening result: (1) engaging in insertive or both insertive and receptive anal intercourse, (2) reporting STI symptoms, and (3) reporting condomless sex.

The STI period prevalence of 33% and the incidence rate of 61.6 per 100 person-years in our study is high compared with other studies in Western Europe. A study of HIV-infected MSM in the SHCS found an overall point STI prevalence of 20%, and another study from Germany reported a markedly lower STI prevalence of 15% in its MSM population [12, 23].

The very high prevalence in our study could be explained by several factors. First, individuals initially diagnosed with primary HIV infection might constitute per se a high-risk population as suggested by our analysis of rates of risky sexual behavior in patients with a diagnosis of primary versus chronic HIV infection. Second, individuals receiving suppressive ART are considered noninfectious for their sexual partners in terms of HIV, which might result in risk-compensation behavior and thus higher risk for contracting bacterial STIs and hepatitis C. In line with this assumption, a 2-fold increase in the syphilis incidence was observed in the SHCS after 2008, the year the so-called Swiss statement was introduced, which declared that HIV-infected persons receiving effective HIV treatment are not

Table 3. Association of Selected Factors With Any Sexually Transmitted Infection (STI) and Asymptomatic STI, Versus No STI

Factor	Overall	No STI	Any STI	*P* Value[a]	Asymptomatic STI	*P* Value[a,b]
Visits, No.	334	263	71	…	46	…
Age at STI diagnosis, median (IQR), y	33.8 (28.2–41.0)	33.8 (28.8–41.6)	33.2 (27.4–36.8)	.28	33.2 (27.4–36.8)	.39
Male sex, %	326 (97.6)	256 (97.3)	70 (98.6)	1	45 (97.8)	1
Time since HIV diagnosis, median (IQR), y	4.9 (2.3–7.9)	5.3 (2.7–8.2)	3.2 (1.5–6.5)	.006	3.3 (1.8–6.4)	.16
Year of HIV diagnosis year, median (IQR)	2010 (2007–2013)	2010 (2007–2013)	2012.0 (2009–2014)	.009	2012 (2008–2013)	.16
White ethnicity, %	311 (93.1)	248 (94.3)	63 (88.7)	.19	41 (89.1)	.36
Higher education, %	75 (22.5)	62 (23.6)	13 (18.3)	.54	10 (21.7)	.88
Risk group MSM, %	305 (91.3)	235 (89.4)	70 (98.6)	.05	45 (97.8)	.23
Stable partner, %	181 (54.2)	150 (57.0)	31 (43.7)	.12	22 (47.8)	.41
Sexual contact, %	321 (96.1)	250 (95.1)	71 (100.0)	.14	46 (100.0)	.39
Anal intercourse, %				.03		.17
No	55 (16.5)	52 (19.8)	3 (4.2)		2 (4.3)	
Receptive	50 (15.0)	37 (14.1)	13 (18.3)		7 (15.2)	
Insertive	33 (9.9)	26 (9.9)	7 (9.9)		6 (13.0)	
Both	196 (58.7)	148 (56.3)	48 (67.6)		31 (67.4)	
Oral sex only, %	18 (5.4)	18 (6.8)	0 (0.0)	.05	0 (0.0)	.23
No. of sexual partners, median (IQR)	2.0 (1.0–4.8)	2.0 (1.0–4.0)	3.0 (2.0–6.5)	<.001	3.5 (2.0–7.8)	.004
Condomless sex, %	131 (39.2)	89 (33.8)	42 (59.2)	.001	24 (52.2)	.16
STI symptoms, %	48 (14.4)	24 (9.1)	24 (33.8)	<.001	0 (0.0)	.16
No. of symptoms, median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	<.001	0.0 (0.0–0.0)	.16
CD4 cell count at STI screen, median (IQR), cells/μL	696.0 (523.0–867.0)	707.0 (525.5–886.0)	605.5 (488.5–795.5)	.09	602.0 (482.5–759.0)	.17
CD4/CD8 cell count ratio, median (IQR)	1.2 (0.9–1.5)	1.2 (0.9–1.5)	1.1 (0.9–1.4)	.14	1.0 (0.8–1.4)	.23
Virally suppressed, %	308 (92.2)	244 (92.8)	64 (90.1)	.54	42 (91.3)	.88
History of syphilis, %	121 (36.9)	92 (35.5)	29 (42.0)	.46	21 (46.7)	.36
History of depression, %	99 (29.7)	81 (30.8)	18 (25.7)	.54	12 (26.1)	.73
Psychiatric history, %	82 (24.6)	71 (27.0)	11 (15.7)	.12	7 (15.2)	.23
Ever smoked, %	173 (52.6)	139 (53.7)	34 (48.6)	.56	21 (45.7)	.49
Any recent drug use, %	44 (13.2)	32 (12.2)	12 (17.1)	.46	6 (13.0)	.88
Binge drinking, %				.54		.57
Never	274 (84.8)	220 (85.9)	54 (80.6)		37 (80.4)	
Monthly or less	37 (11.5)	27 (10.5)	10 (14.9)		6 (13.0)	
Weekly or more	12 (3.7)	9 (3.5)	3 (4.5)		3 (6.5)	
Housing, %				.58		.88
Alone	253 (84.9)	205 (85.8)	48 (81.4)		33 (82.5)	
With friends	37 (12.4)	27 (11.3)	10 (16.9)		6 (15.0)	
Other	8 (2.7)	7 (2.9)	1 (1.7)		1 (2.5)	
Traveled to tropics, %	42 (12.6)	33 (12.5)	9 (12.9)	>.99	8 (17.4)	.49
Physical activity, %				.12		.23
Low	72 (21.6)	64 (24.3)	8 (11.4)		5 (10.9)	
Moderate	154 (46.2)	118 (44.9)	36 (51.4)		23 (50.0)	
High	107 (32.1)	81 (30.8)	26 (37.1)		18 (39.1)	
BMI, median (IQR), kg/m^2	23.5 (21.9–25.7)	23.7 (22.1–26.2)	22.7 (21.7–24.6)	.07	23.2 (21.9–25.6)	.49

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men: STI, sexually transmitted infection.

[a]Benjamini-Hochberg adjustment for variables in the table.

[b]Compared with no STI.

at risk of transmitting HIV through sexual contact [5, 35, 36]. The fact that 3 individuals were identified with acute HCV infection reflects the current HCV epidemic affecting MSM, predominantly those coinfected with HIV, and it emphasizes the need for systematic HCV screening in the affected population [37].

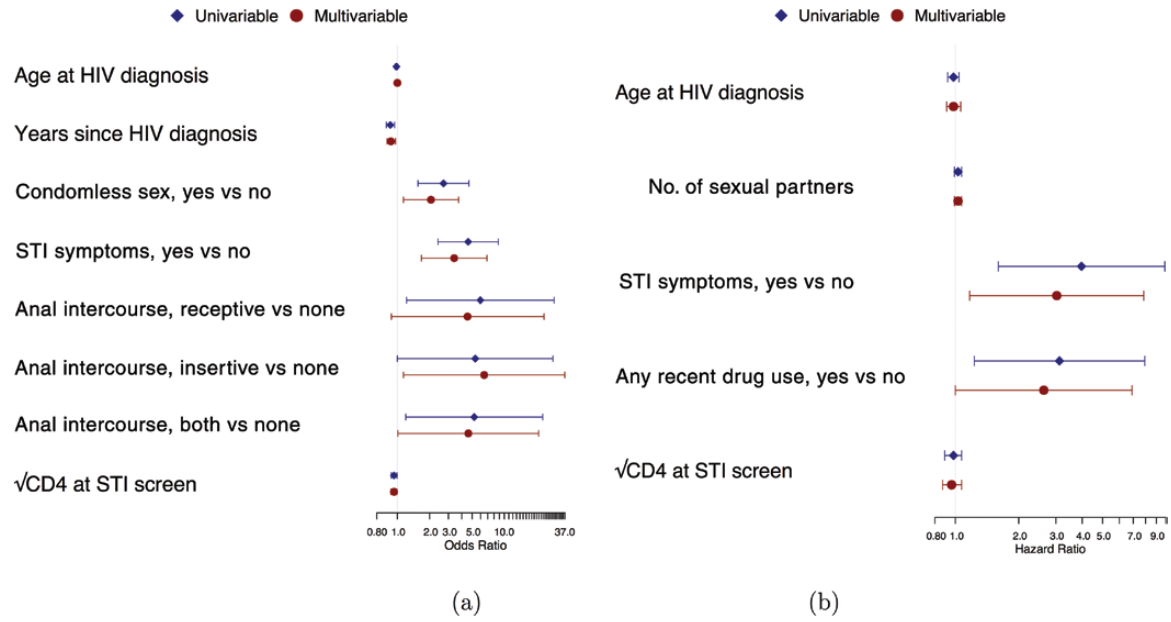

Figure 2. *A*, Mixed model logistic regression for factors associated with a positive sexually transmitted infection (STI) screening (men who have sex with men [MSM] only). *B*, Cox Proportional hazard model for factors associated with incident STI (MSM only). Abbreviations: HIV, human immunodeficiency virus; STI, sexually transmitted infection.

In line with the literature, the majority (66%) of our patients with an STI did not report any symptoms at presentation and immediately before STI testing. In recent years, a plethora of data have been published supporting the strategy to screen asymptomatic sexually active individuals for STIs, especially in the MSM population [4, 8, 9, 11, 13, 23, 28, 30]. Because up to 90% of STIs are asymptomatic [23], a symptom-based testing approach would fail to both accurately detect STIs and interrupt the transmission chain by prompt treatment. On the other hand, and as expected, symptoms were highly predictive of an STI in this high-risk population in the minority of cases in which they were reported.

Table 4. Incident Sexually Transmitted Infections By Self-Reported Drug Use in Past 6 Months (Men Who Have Sex With Men Only)

Self-Reported Drug Use	MSM, No. (%)				
	Overall (n = 68)	No incident STI (n = 47)	Incident STI (n = 21)	*P* Value	Adjusted *P* Value[a]
Any recent drug use	10 (14.7)	2 (4.3)	8 (38.1)	<.001	.003
Intravenous drug use					
Heroin	68 (100.0)	47 (100.0)	21 (100.0)		
Cocaine	68 (100.0)	47 (100.0)	21 (100.0)		
Other	1 (1.5)	0 (0.0)	1 (4.8)	.31	.31
Noninjectable drug use					
Heroin	68 (100.0)	47 (100.0)	21 (100.0)		
Cocaine	5 (7.4)	0 (0.0)	5 (23.8)	.002	.005
Cannabis	4 (5.9)	0 (0.0)	4 (19.0)	.007	.01
Other	9 (13.2)	2 (4.3)	7 (33.3)	.003	.006
Ecstasy	6 (8.8)	0 (0.0)	6 (28.6)	<.001	.003
Gamma-hydroxybutyric acid	4 (5.9)	0 (0.0)	4 (19.0)	.007	.01
Poppers	6 (8.8)	2 (4.3)	4 (19.0)	.07	.08

Abbreviations: MSM, men who have sex with men; STI, sexually transmitted infection.

[a]Benjamini-Hochberg adjustment for variables in the table.

In our multivariable model restricted to MSM, a longer time since HIV diagnosis and a higher √CD4 cell count were associated with lower odds of a positive STI screening result. The negative—age-adjusted—association with the duration of HIV infection might indicate that individuals in whom HIV infection was diagnosed more recently have a higher sensitivity to safe-sex fatigue or a higher rate of STI treatment optimism [38] than those whose HIV infection was diagnosed in previous years. However, this hypothesis should be further investigated in future behavioral studies. The negative association of an STI with √CD4 cell count probably has no clinical significance because the median CD4 cell count even among patients with an STI was relatively high (605/μL). Moreover, a recent study with a large sample size did not find an association of √CD4 cell count with the hazard of contracting syphilis [5].

We found that engaging in anal intercourse, reporting condomless sex, and also reporting any recent drug use for incident STI, predicted a positive STI screening result. All of these variables are a proxy for risky sexual behavior and associated with a higher incidence of STIs in several studies [5, 13, 23, 30, 34]. In line with the literature [13, 25], we found a strong association between incident STI and recent use of noninjectable

drugs, specifically noninjectable cocaine, psychotropic drugs, and cannabis. Although only 1 MSM reported intravenous drug use, we believe that some underreporting cannot be ruled out in our study population. Even though the use of certain drugs such as methamphetamine, mephedrone, and gamma-hydroxybutyrate/gamma-butyrolactone in combination with sex—often referred to as "chemsex"—was initially occurring predominantly in London [39], similar scenes have also set up in other European cities with an established gay culture, including Switzerland [40]. Recently, local clusters of crystal methamphetamine use in rural and urban areas in Switzerland have been reported [41].

Our study findings have several implications for clinical practice. First, a trimonthly STI screening can benefit MSM reporting sexually risky behavior and/or any recent drug use, regardless of symptoms. This is supported both by empirical data and by a modeling study of preexposure prophylaxis users showing that more intensive STI screening might substantially decrease the STI rate [42, 43]. Second, because most but not all diagnosed STIs affected the rectum, the STI screening should include all 3 anatomic sites: rectum, pharynx, and urethra. A pooled STI screening is a promising way to reduce costs and promote acceptance of this approach by health insurers. Third, because the presence of symptoms was highly predictive for a positive STI screening result in our study, symptoms and signs compatible with an STI should trigger the prompt initiation of STI testing. Finally, consultations should be used to promote safer sex practices, because suppressive ART does not replace condoms for protection from STIs in general. However, because all of our patients were counseled accordingly, the effect of additional counseling is questionable.

Our study has several strengths and limitations. One strength is that the prospective and longitudinal study design allowed us to calculate incidence rates. In addition, owing to the unique design of the SHCS and the ZPHI studies, we were able to test associations between STIs and a variety of risk and behavioral factors. One limitation of the study is that, because of the change from a 3-site to a pooled screening approach, we could not assess the exact site of the infection during the whole study. However, this had no implication for the treatment of the STI or for identification of risk factors associated with STI prevalence and incidence.

In conclusion, we found a very high prevalence of asymptomatic STIs in patients who initially presented with a primary HIV-1 infection, most of whom were treated with suppressive ART during the study. The significant associations of STIs with anal intercourse and the reporting of condomless sex or any recent drug use could help identify a subgroup of high-risk MSM and prioritize screening and resource allocations.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The study was designed by D. L. B. and H. F. G. Data acquisition was done by D. L. B., D. S., P. W. S., and C. G. Statistical analysis was performed by A. M., A. U. S., and R. D. K., and H. F. G. and R. D. K. supervised the study. D. L. B. and A. M. wrote the first draft. All investigators contributed to data collection and interpretation of the data, reviewed drafts of the manuscript, and approved the final manuscript.

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CHAPTER VI

"Dietary patterns and physical activity correlate with total cholesterol independently of lipid lowering drugs and ART in aging HIV positive individuals"

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Description of personal contribution

The project was conceptualized by Helen Kovari and AM. HK amd AM wrote the SHCS proposal. AM established, programmed, maintained the RedCap database for the nutrition questionnaires. AM supervised data collection. AM performed several rounds of quality control and performed univariable and multivariable statistical analysis, using various approaches. AM produced all the tables and figures and wrote the first draft and the final version.

Research in context

While current guidelines encourage the implementation of lifestyle changes before statin initiation for cardiovascular disease prevention, empirical data about the association of diet and exercise with total cholesterol in aging HIV positive individuals is absent.

To fill this gap, we interviewed 395 Swiss HIV Cohort participants older than 45 years old, using a validated Food-Frequency-Questionnaire and found that the combined consumption of refined/milled grains, meat, carbonated beverages and coffee exhibited positive association with total cholesterol. On the other hand, regular physical activity, exhibited a negative correlation. Importantly, forty percent of our sample reported very low level or absence of leisure physical activity. In summary, our data shows that physical activity can be substantially scaled up in this population. The reported dietary patterns carry an important prevention potential to achieve cardiovascular and other health benefits in HIV positive persons.

Open Forum Infectious Diseases

MAJOR ARTICLE

Dietary Patterns and Physical Activity Correlate With Total Cholesterol Independently of Lipid-Lowering Drugs and Antiretroviral Therapy in Aging People Living With Human Immunodeficiency Virus

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Background. Hypercholesterolemia is a well established risk factor for coronary heart disease and is highly prevalent among human immunodeficiency virus (HIV)-positive persons. Antiretroviral therapy (ART) can both directly modify total cholesterol and have drug-drug interactions with statins. This makes investigating modifiable behavioral predictors of total cholesterol a pertinent task.

Methods. To explore the association between diet and physical activity with cross-sectionally measured total cholesterol, we administered a validated Food-Frequency-Questionnaire to participants of the Swiss HIV Cohort Study ≥45 years old. Linear mixed-effects models were applied to explore the associations between dietary patterns and physical activity with total cholesterol, after adjustment for clinical and demographic covariates.

Results. In total, 395 patients were included. Forty percent (158 of 395) had elevated total cholesterol (>5.2 mmol/L), and 41% (164 of 395) were not regularly physically active. In multivariable analysis, 2 factors were positively associated with total cholesterol; female sex (β = 0.562; 95% confidence interval [CI], 0.229–0.896) and the combined consumption of meat, refined/milled grains, carbonated beverages, and coffee (β = 0.243; 95% CI, 0.047–0.439). On the other hand, regular physical activity (β = −0.381; 95% CI, −0.626 to −0.136), lipid-lowering drugs (β = −0.443; 95% CI −0.691 to −0.196), ART containing tenofovir (β = −0.336; 95% CI −0.554 to −0.118), and black ethnicity (β = −0.967; 95% CI −1.524 to −0.410) exhibited a negative association.

Conclusions. We found independent associations between certain dietary patterns and physical activity with total cholesterol. Increasing physical activity might achieve cardiovascular and other health benefits in HIV-positive individuals. The clinical relevance of the identified dietary patterns requires further investigation in prospective cohort studies and randomized controlled trials.

Keywords. aging; diet; HIV; physical activity; total cholesterol.

Although life expectancy in human immunodeficiency virus (HIV)-positive people on antiretroviral therapy (ART) has improved worldwide in recent years, it remains shorter in comparison with the general population [1].

Total cholesterol is a well established and low-cost marker of coronary heart disease (CHD), even across cultures [2]. A recent systematic review and meta-analysis showed that total cholesterol is a strong risk factor for CHD in the general population, with 1-mmol/L increase in total cholesterol associated with 20% higher risk of CHD in women and 24% in men [3].

Both HIV infection per se and its treatment may be associated with high total cholesterol [4]. Certain ART agents are associated with a range of metabolic abnormalities—including HIV lipodystrophy, dyslipidemia, diabetes mellitus, and insulin resistance [5]—and contribute to the elevated risk of cardiovascular disease (CVD) [6]. In addition, individuals who are HIV positive generally tend to have a higher rate of traditional risk factors for CVD such as smoking and hyperlipidemia [7].

Although statins can significantly reduce total cholesterol, their use might present a challenge for HIV-positive patients due to drug-drug interactions with ART [8] and in light of the main treatment goal to achieve viral suppression, which often requires change of regimen. Moreover, older patients who are HIV positive have an elevated risk of polypharmacy [9].

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All this makes understanding the contribution of behavioral factors like dietary habits and physical activity a pertinent task which carries a possible prevention potential [10, 11], because both are modifiable risk factors. Although current guidelines encourage the implementation of lifestyle changes before statins initiation for CVD prevention [12], no empirical data about the association of diet and exercise with total cholesterol in aging HIV-positive individuals are available.

Therefore, the main aims of this study were as follows: (1) describe the predictors of total cholesterol in an aging HIV-positive population living in a resource-rich setting; (2) describe the major dietary patterns of this population; (3) and examine whether behavioral determinants such as dietary patterns and physical activity independently correlate with total cholesterol after adjustment for highly active ART, lipid-lowering drugs, and other confounders.

METHODS

Swiss Human Immunodeficiency Virus Cohort Study

The Swiss HIV Cohort Study (SHCS) is a large, ongoing, multicenter cohort study of HIV-positive individuals with a prospective recruitment since 1988 [13]. During the biannual outpatient visits, comprehensive clinical and behavioral data (including leisure physical activity) are collected. The patients described in this study are all enrolled in the SHCS nested project "Metabolism and Aging", which includes participants ≥45 years of age undergoing dual energy x-ray absorptiometry scan, neurocognitive testing, and in the 2 centers of Zurich and Geneva additionally coronary computed tomography scan. For this project, Metabolic and Aging study participants of the Zurich, Berne, and Geneva centers were enrolled. Ethical approval of the SHCS and written informed consent from all participants were obtained upon cohort enrollment.

Dietary Assessment

Information on diet was obtained by a short and validated Food-Frequency Questionnaire (FFQ). This qualitative FFQ was originally used in the large INTERHEART study conducted in 52 countries [14] and was administered thereafter in several other large international studies [15, 16]. In this questionnaire, participants are asked, "In the last 12 months, how often did you consume foods from each of the following categories?" A list of 23 broad food categories is then presented, and the subject states the number of consumed items per month, week, or day. Because this FFQ was designed for use in international studies, it contains all of the main food groups, ie, dairy, meat, fish, fruits, and vegetables, and has been found to be applicable to different countries despite regional dietary differences [14]. Although we intentionally kept the original questionnaire intact to maintain its validity, we added a short supplementary part that gathered additional information about the following: (1) dietary supplements (vitamins, minerals, etc); (2) coffee and tea consumption; and (3) major dietary change in the last 2 years. Data about alcohol consumption are regularly gathered in the SHCS. The FFQ interviews were conducted by phone by a trained staff and were collected using the REDCap electronic data capture tool [17] hosted at University Hospital Zürich.

Statistical Analysis

To assess the dietary patterns, first, all food items were converted into daily consumption. Then, Ward error sum of squares hierarchical clustering method [18, 19] was used with 1-Kendall's tau correlation matrix as an input. Based on the classification output, the 4 largest and most general food clusters were extracted, and for each person the sum of the consumption frequency of food items that belong to each of the 4 clusters was calculated separately. In other words, each person received 4 score variables (pattern I to pattern IV) that reflected the cumulative consumption frequency of the food items for each pattern.

Linear mixed-effects model with per center random intercept was used to assess the correlation between dietary patterns and total cholesterol. The closest available total cholesterol measurement to the dietary assessment was used regardless of fasting state. Fasting state during the measurement was adjusted for in the multivariable models. Two multivariable models were explored: (1) the minimally adjusted model, only for age, sex, risk group, physical activity, ART, lipid-lowering drugs, and fasting state; and (2) the model that was adjusted for the covariates in model I plus additional demographical and clinical covariates (ethnicity, university education, living alone, smoking, depression, diabetes, hypertension, number of years on ART, recent dietary change, multivitamin supplement, Omega 3 supplement, protein powder supplement) (Figure 4). Because the cumulative consumption scores were skewed to the right, they were ln(x+1) transformed. Benjamini-Hochberg adjustment for multiple testing was performed for the correlation matrix (Figure 2) but not for the multivariable analysis, due to limited power. Statistical analysis was performed with R (version 3.3.2).

RESULTS

Patients

In total, from June 2016 until October 2017, of 539 eligible participants 395 (73.3%) were interviewed for the study, the remaining either refused to participate (n = 31, 5.8%), could not be reached on at least 5 different occasions (n = 111, 20.6%), or provided incomplete response (n = 2). The median age of the participants was 55.7 years (interquartile range [IQR], 52.2–60.8), the majority were male (85.6%, 338 of 395), of white ethnicity (94.4%, 373 of 395), and belonged to the men-who-have-sex-with-men (MSM) risk group (59%, 233 of 395) (Table 1). A total of 11.6% (46 of 395) were obese and 32.4% (128 of 395) were overweight. The median time on ART was 14.2 years (IQR, 8.8–20.4), and the median most recent CD4 value was 648 cells/μL (IQR, 494.5–816.0). The vast majority of patients (95.9%, 379

Table 1. Patient Characteristics

Characteristics	Overall
n	395
Age (median [IQR])	55.7 [52.2–60.8]
Sex, female (%)	57 (14.4)
Ethnicity (%)	
White	373 (94.4)
Black	15 (3.8)
Hispanic	7 (1.8)
Risk Group (%)	
Heterosexual	108 (27.3)
Men who have sex with men	233 (59.0)
Injecting drug users	24 (6.1)
Other	30 (7.6)
University education, yes (%)	55 (13.9)
Living alone, yes (%)	162 (41.0)
Current smoking, yes (%)	126 (31.9)
BMI (%)	
Normal (≥18.5–25)	212 (53.7)
Overweight (≥25–30)	128 (32.4)
Obese (≥30)	46 (11.6)
Underweight (<18.5)	9 (2.3)
Depression, yes (%)	59 (14.9)
Diabetes, yes (%)	23 (5.8)
Hypertension, yes (%)	135 (34.2)
Lipid-lowering drugs, yes (%)	98 (24.8)
Virally suppressed, yes (%)	379 (95.9)
CD4 (median [IQR])	648.0 [494.5–816.0]
Years on ART (median [IQR])	14.2 [8.8–20.4]
On NRTI, yes (%)	320 (81.0)
On NTRTI, yes (%)	192 (48.6)
On NNRTI, yes (%)	117 (29.6)
On PI, yes (%)	107 (27.1)
On INTI, yes (%)	227 (57.5)
Physical activity (%)	
Never	124 (31.4)
1–4 times a month	40 (10.1)
1–2 times a week	81 (20.5)
≥3 times a week	150 (38.0)
Dietary Change in the Last 2 Years (%)	
No	360 (91.1)
Became vegetarian	3 (0.8)
Other change	32 (8.1)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; INTI, integrase inhibitor; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NTRTI, nucleotide reverse-transcriptase inhibitor; PI, protease inhibitor.

of 395) were virally suppressed with HIV viral load below 50 copies/mL.

Dietary Patterns

First, we aimed to explore which food groups correlate and cluster with each other. In other words, when patients consume one food group, which other food groups they are likely to consume as well (positive association) or on the other hand to avoid (negative association). Hierarchical clustering analysis suggested 4 distinct metapatterns (Figure 1, Figure 2): (Pattern I) "Meat," "Refined/ milled grains," "Carbonated beverages," "Coffee" - This pattern positively correlated with smoking, with 21.4% (22 of 103) of smokers in the lowest quartile and 45.8% (44 of 96) of smokers in the highest quartile (Fisher's exact test, $P < .001$) (Supplementary Table 1); (Pattern II) "Organ meats," "Poultry," "Fish/seafood," "Alcohol" - Pattern II negatively correlated with female sex, with 26.7% (27 of 101) females in the lowest quartile and 8.2% (8 of 97) in the highest consumption quartile (Fisher's exact test, $P = .001$) (Supplementary Table 2); (Pattern III) "Whole grains," "Dairy products," "Eggs," "Leafy green vegetables," "Other vegetables (raw)," "Other vegetables (cooked)," "Legumes/nuts/seeds," "Potatoes, boiled/mashed," "Pickled food," "Fruits," "Tea (Black/Green)" - Pattern III was positively associated with university education with 9.1% (9 of 99) having university education in the lowest quartile versus 22.2% (22 of 99) in the highest (Fisher's exact test, $P = .018$) and was negatively associated with smoking with 45.5% (45 of 99) smokers in the lowest quartile and 20.2% (20 of 99) in the highest consumption quartile (Fisher's exact test, $P < .001$) (Supplementary Table 3); (Pattern IV) "Pizza," "Deep fried foods," "Salty snacks," "Ice cream/pudding," "Desserts/sweet snacks," "Confectionary sugars/syrups," "Fruit juice/drinks" - This pattern was negatively associated with diabetes, hypertension, and lipid-lowering drugs (Fisher's exact test, $P = .016$, .011, and .014, respectively), suggesting that patients diagnosed with these conditions tend to avoid junk food (Supplementary Table 4). Noteworthy pairwise negative associations (Figure 2) were as follows: patients who consumed fruits more frequently consumed less frequently meat, refined/milled grains, and carbonated beverages. Coffee and tea were also negatively correlated (Kendall's tau −0.19, adjusted $P < .001$), suggesting that when it comes to regular consumption, some patients tend to prefer either one or the other. Next, we explored separately the frequency of meat consumption; only 3.3% (13 of 395) of the participants reported to completely avoid meat and organ meats, and only 5 patients were lacto-ovo-vegetarian (1.3%, 5 of 395) avoiding meat, organ meats, poultry, and fish.

Examining the total daily consumption frequency stratified by pattern (Figure 3) shows that the most frequently consumed pattern is the apparently healthier pattern III (mean = 6.5, standard deviation [sd] = 3.3), followed by pattern I (mean = 4.6, sd = 3.1), pattern IV (mean = 1.8, sd = 1.1), and pattern II (mean = 0.7, sd = 0.4).

Physical Activity

Thirty-one percent (124 of 395) of the patients reported not to perform any leisure physical activity. Ten percent (40 of 395) reported physical activity frequency of 1 to 4 times a month, 21% (81 of 395) reported physical activity 1 to 2 times a week, and the largest group (38%, 150 of 395) reported physical activity 3 or more times per week.

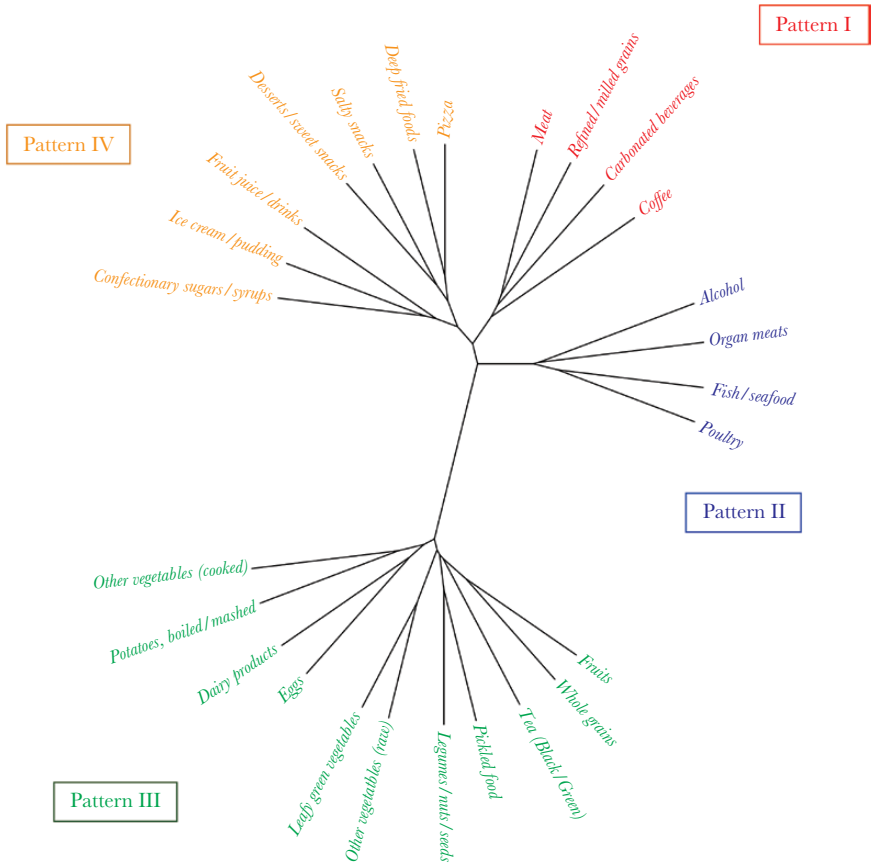

Figure 1. Four main dietary patterns as determined by hierarchical clustering.

Total Cholesterol

The median total cholesterol value was 5 mmol/L (sd = 1.1). Forty percent (158 of 395) of participants were above the cutoff of 5.2 mmol/L, and 13.1% (51 of 395) were above the cutoff of 6.2 mmol/L (Supplementary Figure 1). Lipid-lowering drugs were prescribed in 24.8% of the participants (98 of 395).

Factors Associated With Total Cholesterol

Next, we examined the factors that correlate with total cholesterol. In the first multivariable model (Model I; Figure 4), physical activity at least 3 times a week compared with none (β = −0.274; 95% confidence interval [CI], −0.519 to −0.029), lipid-lowering drugs (β = −0.458; 95% CI, −0.703 to −0.212), ART including a nucleotide reverse-transcriptase inhibitor (NtRTI) (β = −0.344; 95% CI, −0.565 to −0.123), and intravenous drug users (IDU) risk group (β = −0.705; 95% CI, −1.168 to −0.242) were negatively correlated with total cholesterol. On the other hand, female sex (β = 0.487; 95% CI, 0.147 to 0.827) and dietary pattern I ("Meat," "Refined/ milled grains," "Carbonated beverages," "Coffee")—but not the other 3 patterns—positively correlated with total cholesterol (β = 0.276; 95% CI, 0.082–0.471; P = .007).

After additionally adjusting for a wide range of demographic and clinical covariates (Model II; Figure 4), lipid-lowering drugs (β = −0.443; 95% CI, −0.691 to −0.196), NtRTI (β = −0.336; 95% CI, −0.554 to −0.118), and regular physical activity (β = −0.381; 95% CI, −0.626 to −0.136) remained negatively correlated with total cholesterol, in addition to black ethnicity (β = −0.967; 95% CI, −1.524 to −0.410), IDU risk group (β = −0.708; 95% CI, −1.175 to −0.241), living alone (β = −0.236; 95% CI, −0.436 to −0.035), and taking a multivitamin (β = −0.378; 95% CI, −0.730 to −0.027). On the other hand, female sex (β = 0.562; 95% CI, 0.229–0.896) and dietary pattern I (β = 0.243; 95% CI, 0.047–0.439; P = .02) remained positively and significantly correlated with total cholesterol even after adjustment for the additional covariates in Model II.

Sensitivity Analysis

As a sensitivity analysis, we repeated the multivariable analysis (Model I excluding lipid-lowering drugs) separately for patients on and off lipid-lowering drugs (Supplementary Table 5). For patients on lipid-lowering drugs, frequent physical activity (β = −0.732; 95% CI, −1.166 to −0.232), NtRTI (β = −0.546; 95% CI, −0.980 to −0.076), and IDU risk group (β = −1.148; 95% CI, −2.023 to −0.278) negatively correlated with total cholesterol. None of the covariates, including dietary pattern I (β = 0.352; 95% CI, −0.039 to 0.747; P = .115), significantly positively correlated with total cholesterol among patients on lipid-lowering

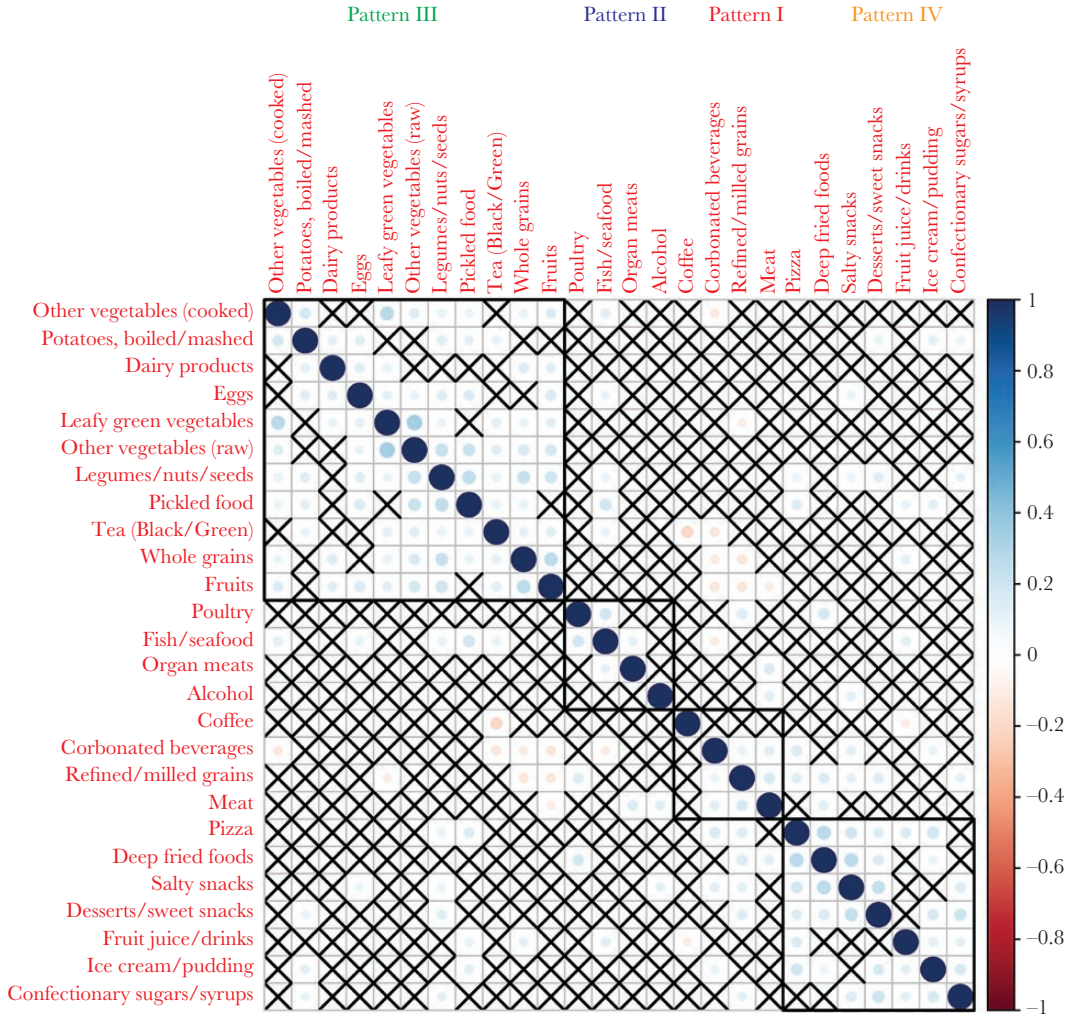

Figure 2. Correlation matrix of the examined food items. Squares with "X" are not statistically significant after adjustment for multiple testing.

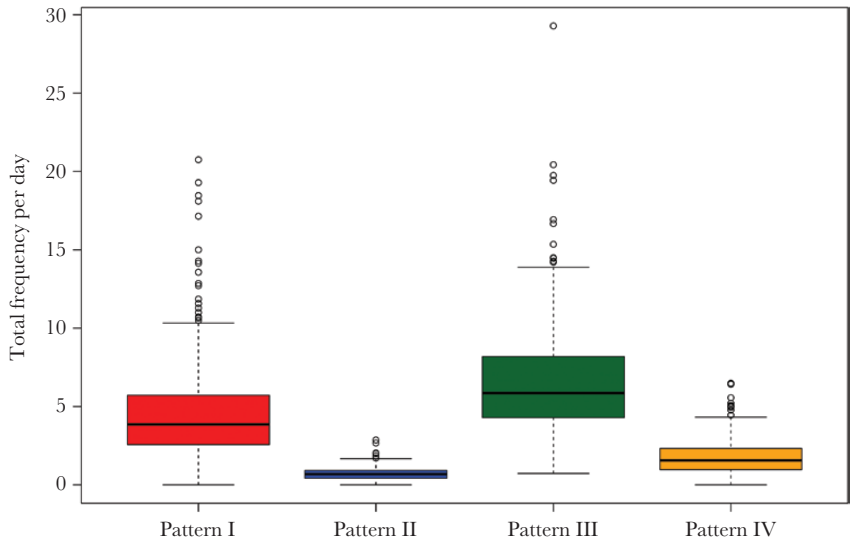

Figure 3. Boxplot of total daily consumption stratified by dietary patterns. Pattern I: meat, refined/milled grains, carbonated beverages, coffee. Pattern II: organ meats, poultry, fish/seafood, alcohol. Pattern III: whole grains, dairy products, eggs, leafy green vegetables, other vegetables (raw and cooked), legumes/nuts/seeds, potatoes, boiled/mashed, pickled food, fruits, tea (black/green). Pattern IV: pizza, deep fried foods, salty snacks, ice cream/pudding, desserts/sweet snacks, confectionary sugars/syrups, fruit juice/drinks.

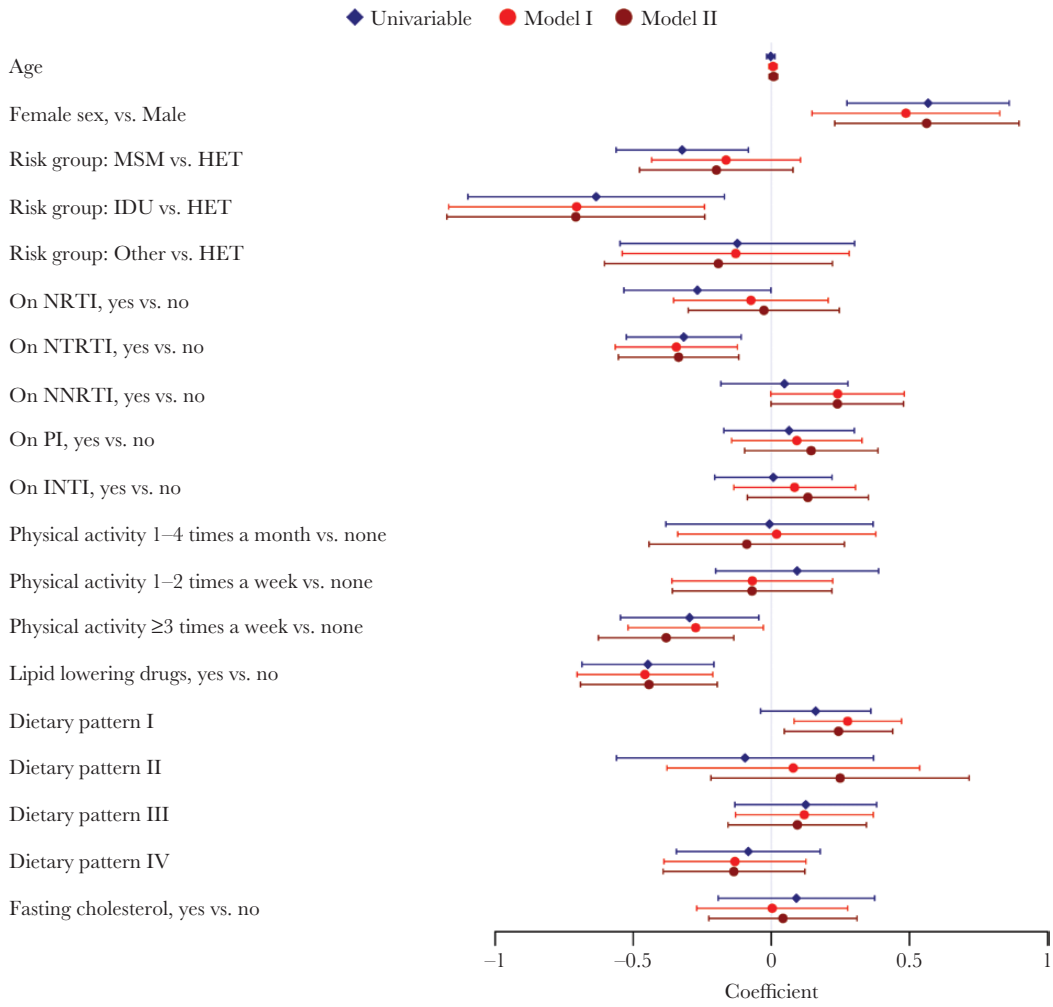

Figure 4. Factors that correlate with total cholesterol, univariable and 2 linear mixed-effect models. Model I was adjusted for all the covariates in the figure. Model II was adjusted for all covariates in the figure and additionally for ethnicity, university education, living alone, smoking, depression, diabetes, hypertension, number of years on antiretroviral therapy, recent dietary change, multivitamin supplement, Omega 3 supplement, protein powder supplement. Dietary patterns consumption frequencies were ln(x+1) transformed. Pattern I: meat, refined/milled grains, carbonated beverages, coffee. Pattern II: organ meats, poultry, fish/seafood, alcohol. Pattern III: whole grains, dairy products, eggs, leafy green vegetables, other vegetables (raw and cooked), legumes/nuts/seeds, potatoes, boiled/mashed, pickled food, fruits, tea (black/green). Pattern IV: pizza, deep fried foods, salty snacks, ice cream/pudding, desserts/sweet snacks, confectionary sugars/syrups, fruit juice/drinks. Abbreviations: ART, antiretroviral therapy; HET, heterosexual; IDU, injecting drug users; INTI, integrase inhibitor; MSM, men who have sex with men; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NTRTI, nucleotide reverse-transcriptase inhibitor; PI, protease inhibitor.

drugs; however, the limited statistical power of this subgroup analysis should be taken into account.

Among patients not on lipid-lowering drugs, none of the covariates were significantly negatively associated with total cholesterol. On the other hand, dietary pattern I and female sex remained significantly positively correlated with total cholesterol in people not on lipid-lowering drugs (β = 0.234, 95% CI = 0.011–0.457, P = .047 and β = 0.574, 95% CI = 0.198–0.949, P = .004, respectively).

High-Density Lipoproteins and Triglycerides

Finally, to obtain a more complete picture on factors associated with dyslipidemia in general, we also explored the fully adjusted model with high-density lipoproteins (HDL) and triglycerides as outcomes. For HDL, age (β = 0.012; 95% CI, 0.006–0.019) and female sex (β = 0.474; 95% CI, 0.331–0.615) showed a positive association, whereas having hypertension (β = −0.132; 95% CI, −0.227 to −0.036) and ART regimen with protease inhibitors (β = −0.121; 95% CI, −0.224 to −0.018) exhibited a negative correlation. None of the remaining variables, including physical activity and the examined 4 dietary patterns, showed any significant correlation. For triglycerides, diabetes (β = 0.730; 95% CI, 0.144–1.367), hypertension (β = 0.371; 95% CI, 0.045–0.690), nonnucleoside reverse-transcriptase inhibitors (β = 0.480; 95% CI, 0.131–0.822), protease inhibitors (β = 0.695; 95% CI, 0.342–1.038), and integrase inhibitors (β = 0.589; 95% CI, 0.259–0.891) showed positive association, whereas age (β = −0.024; 95% CI, −0.045 to −0.001), female sex (β = −0.554; 95% CI,

−1.009 to −0.047), MSM risk group (β = −0.455; 95% CI, −0.836 to −0.036), IDU risk group (β = −1.213; 95% CI, −1.886 to −0.540), living alone (β = −0.332; 95% CI, −0.630 to −0.051), and physical activity of at least 3 times per week (β = −0.389; 95% CI, −0.764 to −0.058) were negatively associated with triglycerides. In contrast to physical activity, none of the dietary patterns exhibited a significant association with triglycerides in our fully adjusted model.

DISCUSSION

In this analysis of HIV-positive individuals ≥45 years old predominantly on suppressive ART, we found several factors that were independently correlated with total cholesterol: female sex and the frequency of combined consumption of meat, refined/milled grains, carbonated beverages, and coffee (dietary pattern I) showed positive correlation. On the other hand, regular physical activity, lipid-lowering drugs, an ART regimen containing tenofovir, and black ethnicity exhibited a negative correlation. In the analysis restricted to patients not on lipid-lowering drugs, total cholesterol remained associated with dietary pattern I (meat, refined/milled grains, carbonated beverages, coffee), supporting current guidelines that encourage implementation of lifestyle and dietary changes before statin initiation [12]. In participants on lipid-lowering drugs, there was an additional benefit of physical activity, beyond the effect of lipid-lowering drugs.

It has been previously shown that people living with HIV consume more saturated fat and cholesterol compared with HIV-negative individuals [20]. It was also demonstrated that tackling HIV dyslipidemia with diet is feasible [10]. In a systematic review and meta-analysis of the effects of dietary intervention on HIV dyslipidemia, dietary intervention reduced total cholesterol by −0.36 mmol/L compared with placebo/control [21]. However, no study was performed on a predominantly aging HIV-positive population.

Several previous studies in the general population demonstrated associations between individual food items that comprise pattern I in our study and total cholesterol or cardiovascular risk. In a prospective cohort study of 31 546 high-risk individuals from 40 countries, meat, poultry, and egg consumption were independently correlated with composite outcome of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for congestive heart failure [15]. Although in our analysis meat indeed clustered in pattern I, poultry and eggs clustered in different patterns.

Our results are biologically plausible because most meat products contain saturated fat that increases cholesterol [22]. In addition, almost all meat products contain cholesterol by itself [23, 24]. Frequency of coffee consumption also clustered with pattern I and is also supported by previous works. In a meta-analysis of 14 clinical trials, a dose-response relation between coffee consumption and both total cholesterol and low-density lipoprotein cholesterol was identified [25]. The effect was weak for filtered coffee; however, most of the coffee that is consumed in Switzerland is unfiltered.

The observation that pattern III that contained vegetables, legumes, and fruits positively correlated with higher education and negatively correlated with smoking is consistent with the well established association between education and health literacy [26]. This might also suggest that patients with low education could benefit most from dietary counseling.

The beneficial effect of regular exercise on cholesterol is well established. For example, in a randomized controlled trial from the UK, in which participants were asked to maintain their normal dietary habits, high-intensity exercise program for 24 weeks resulted in significant decreases in total cholesterol (mean change, −0.55 ± 0.81 mmol/L) [27]. Forty percent of our sample reported physical activity of 1 to 4 times a month or never, hence not meeting the World Health Organization (WHO) guidelines of 150 minutes of moderate-intensity aerobic physical activity throughout the week [28]. This suggests that there is considerable room for improvement in this area, especially because the benefits of physical activity extend beyond cardiovascular risk factors and also include prevention of dementia [29], depression, and anxiety [30]. Data about the benefits of physical activity for HIV dyslipidemia is scarce [31], especially in aging HIV-positive population.

The strengths of our study include the use of a population-based cohort, the SHCS, with a large array of prospectively collected clinical and laboratory data, enabling us to adjust for many important clinical and demographic potential confounders. Patients from 3 different study centers across Switzerland were included, hence increasing generalizability. Moreover, nutrition data were assessed by a well validated questionnaire used in large studies in the general population.

Our study also has limitations. Inherent to cross-sectional studies, there is a lack of temporality [32]. Due to the observational study design, and in light of the fact that there are many potential confounders associated with dietary patterns, physical activity and total cholesterol levels, we cannot rule-out residual confounding for which we could not account. Another limitation is that the FFQ measured frequency but not quantity of consumption [15]. Finally, due to collinearity between food items within the same consumption pattern, it was not possible to examine the effect of single food items. Nevertheless, our results are in line with large cohort studies, randomized controlled trials, and meta-analysis [15, 25, 27].

Future studies are needed to illuminate the effect of type of fat intake (saturated fat, monounsaturated fat, and polyunsaturated fat) on dyslipidemia and on the association between dietary patterns and hard endpoints such as cardiovascular morbidity and mortality. The most beneficial type of physical activity in terms of adherence and cardiovascular benefit for this population is also yet to be determined.

CONCLUSIONS

In summary, our cross-sectional study suggests that there are independent associations between certain dietary patterns and physical activity with total cholesterol. Physical activity should be substantially scaled up in this population to meet WHO guidelines and to achieve cardiovascular and other health benefits. The reported dietary patterns pave the way for further investigations in prospective cohort studies and randomized controlled trials.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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CONCLUSION

In 2016, for the first time, the total number of HIV cases in the WHO European Region surpassed two million. According to the latest WHO/ECDC report 153,407 people were newly diagnosed with HIV in 50 countries of the Region in 2015[1]. However, the epidemic drivers remain different in Western and in Eastern parts of Europe. In Western Europe, the epidemic is continued to be fueled by Men-who-have-Sex-with-Men. In this thesis, we could shed light on the importance of transmission during recent infection and after treatment interruption among Swiss MSM. We also critically characterized the prescription practices of Post-Exposure-Prophylaxis, which is commonly used by MSM, in a study that might improve future prevention. In contrast to Western Europe, the main driver of transmission in Eastern Europe are people-who-inject-drugs. Astonishingly, the number of AIDS cases in this area increased by 80% in 10 years[2]. Since Switzerland experienced an epidemic of opioid addiction in the 1980s, we could gain insights that might be potentially relevant to HIV prevention in Eastern Europe as well, by demonstrating the essential role that harm reduction played in HIV containment both among injecting-drugs-users and the general population. This thesis did however not only concentrate on the prevention of HIV infection, but also on improving the health of people that already live with the virus. This was done on two main levels: (i) We showed high rates of asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection, found epidemiological risk factors for STIs, and suggested that screening might have to be intensified. (ii) We described nutrition and physical activity habits of aging people living with HIV and correlated this data with total cholesterol with the intention to facilitate prevention of coronary heart disease.

In summary, in this thesis, we intended to contribute to various aspects of HIV epidemiology and clinical practice, with a hope to improve prevention efforts and treatment.

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Supplementary Material: Chapter I: *"HIV-1 Transmission During Recent Infection and During Treatment Interruptions as Major Drivers of New Infections in the Swiss HIV Cohort Study"*

Supplementary Text I

For each SHCS participant a seroconversion date (infection date) was estimated based on the following hierarchical algorithm:

(i) The top priority was given to the SHCS participants who were also members of the Zurich Primary HIV Infection Study (ZPHI). For these patients the estimated date of infection as defined in the ZPHI was used as the infection date (see [1, 2] for the definition of this date).

(ii) Provided the patient was not a member of the ZPHI (which was the case for 98.4% of the SHCS participants) the next priority was given to the documented primary infection as recorded by the physicians in the SHCS. In this case, the date of primary infection diagnosis was used as seroconversion date. Primary infection diagnosis by the physicians in the SHCS was based on one of the following criteria: 1. Exposure to HIV within the last three months and clinical documentation or history of signs and/or symptoms suggestive of acute retroviral syndrome (such as acute febrile mononucleosis like syndrome with lymphadenopathy or rash or pharyngitis or aphthous ulceration or meningo encephalitis or myalgia or hepatitis) and a documented negative HIV antibody test within the last six months and detectable HIV-RNA in serum or plasma. 2. Detectable p24 Antigen or HIV-RNA and a negative or borderline HIV antibody test converting to reactive.

(iii) If no data was available to fulfill conditions (i) and (ii) the midpoint date between the last HIV negative date and the earliest HIV positive date was calculated provided that this time interval did not exceed three years (to reduce misclassification bias).

(iv) Finally, if there was no data available to fulfill conditions (i), (ii), (iii) but there was an available, therapy naïve, genotypic drug resistance test for the patient of interest, then the fraction of the ambiguous nucleotides in the earliest sequences was calculated. Previous work has shown that most HIV infections are established by a single virus which subsequently diversifies [3]. Based on this, Kouyos *et al.* [4] showed that the percentage of ambiguous nucleotides (i.e. other than A/T/G/C) in the genotypic resistance test sequences, positively correlates with the disease progression stage. Hence, based on previous studies we have chosen the cutoff of

0.5% for distinction between recent and chronic phase of the infection [4]. To reduce misclassification bias, this was done only for patients for whom the first CD4 count value was above 250 and the time interval between the first sequence and HIV diagnosis was lower than three years. In this case the time of HIV diagnosis was used as a proxy for the seroconversion date.

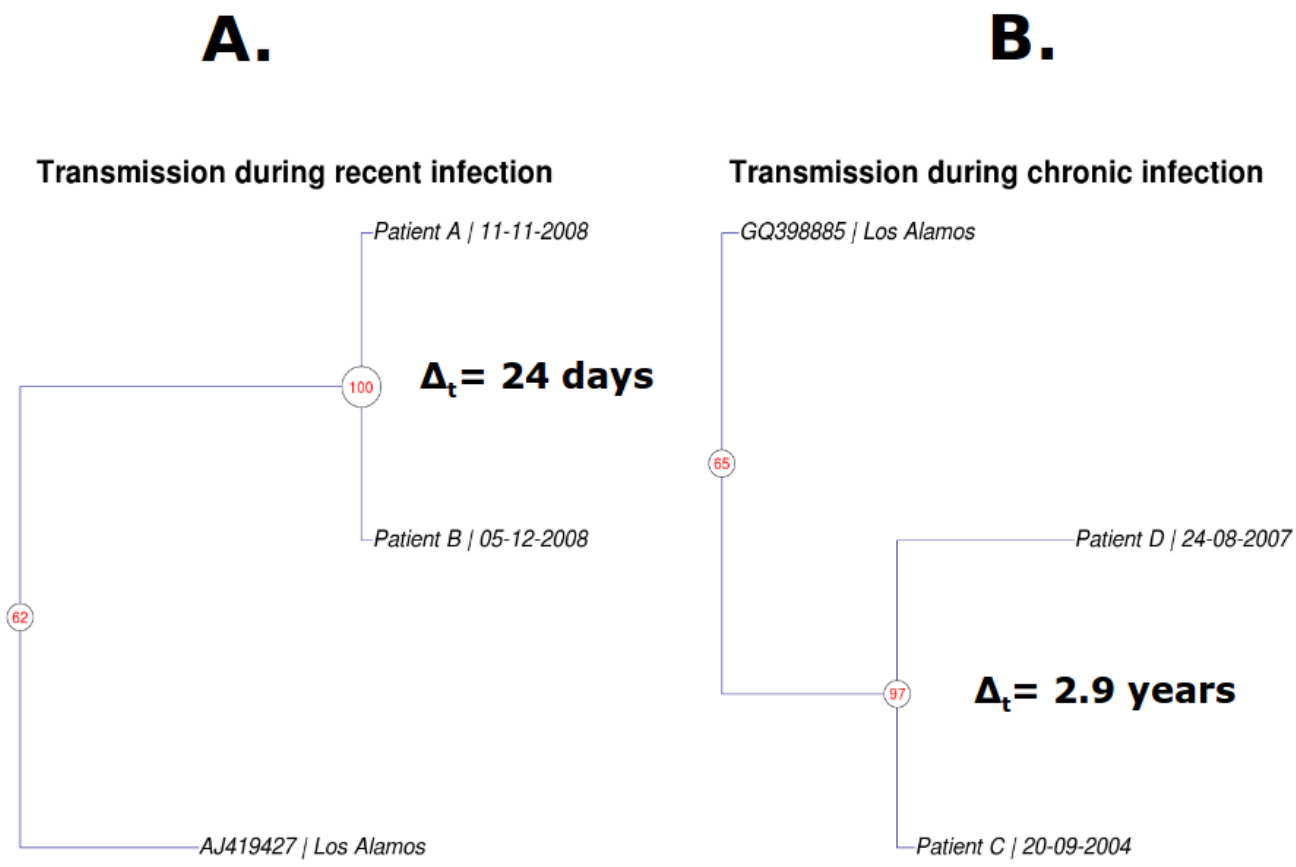
Supplementary Text II

Phylogenetic tree construction

The phylogenetic maximum-likelihood Generalized Time-Reversible (GTR) model based tree was constructed with an overall of 110,598 HIV-1 nucleotide *pol* open-reading-frame protease and reverse-transcriptase (RT) sequences. These were comprised of 19,604 sequences from 10,970 different SHCS cohort participants and additional 90,994 sequences from the Los Alamos (http://www.hiv.lanl.gov/) database which were included to decrease the chances of a false-positive random clustering. All the available, non-Swiss, *pol* sequences (region: 2253-3870) with a minimal length of 900 bp as to September 2014 were retrieved. Redundant control sequences (different sequence id but an identical nucleotide composition) were deduplicated. For the SHCS patients only sequences with a minimum length of 250 bp for the protease gene and 500 bp for the RT gene were included.

All the sequences were initially aligned to a HXB2 reference genome (http://www.ncbi.nlm.nih.gov/nuccore/K03455.1) using the MUSLCE [5]. Next, insertions relatively to HXB2 and resistance mutations according to Stanford: http://hivdb.stanford.edu/ and International AIDS Society https://www.iasusa.org/ lists were removed. In the following step, one hundred maximum-likelihood GTR-model-based bootstrapped trees were constructed using SEQBOOT[6] and FastTree[7]. Finally, node support values were generated based on 100 bootstrapped trees and the reference (non-bootstrapped alignment) tree using the "CompareToBootstrap**"** script. Following the tree construction, tips that corresponded to various pairwise minimal patristic genetic distance (1%, 1.5%, 2%, 2.5%) and the bootstrap (50% to 100% by increment of 2%, of the deepest node) thresholds were defined as potential transmission pairs and were extracted using custom scripts. The R package "APE" version 3.1 was implemented for tree exploration and analysis [8].

Supplementary Figure 1. Example of potential transmission events during recent (panel A) and chronic (panel B) HIV infection as represented on the phylogeny. The tips of the tree represent HIV sequences from different patients, SHCS patients are designated by A-D letters. The numbers at the tree nodes represent the bootstrap percent (red) for each node. Δ_t represents the time interval between the seroconversion dates of the pair members. The cophenetic genetic distance within pair A-B and C-D is 0.1% and 0.6%, respectively.

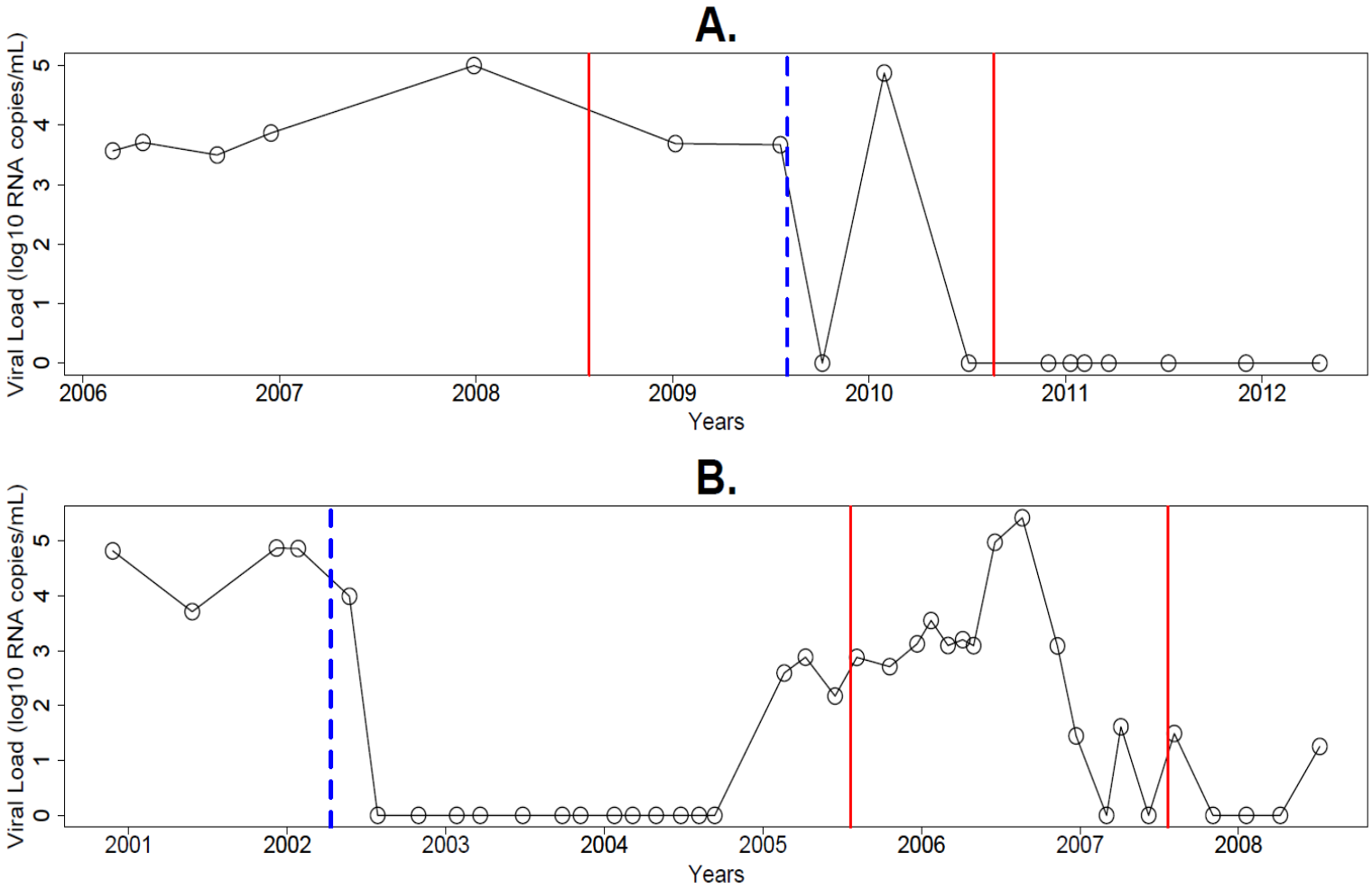


Supplementary Table 1. Logistic regression analysis for being chronic (>1 year since seroconversion, coded as 1) versus recent (<=1 year since seroconversion, coded as 0) phylogenetically linked HIV transmitter. N=255 transmitters (complete cases, 144 chronic and 111 recent), Bootstrap 50%, genetic distance 1.5%. Abbreviations: C.I, confidence interval; OR, odds ratio; ART, antiretroviral therapy; VL AUC, viral load area under the curve.

	Bivariate OR (95% C.I)	P-value	Multivariable OR (95% C.I)	P-value
Age at infection	0.98 (0.95-1)	0.06	0.99 (0.96-1.02)	0.531
Sex				
Male (Ref.)				
Female	1.13 (0.58-2.2)	0.721	1.46 (0.51-4.15)	0.48
Risk group				
Men who have sex with men (Ref.)				
Heterosexuals	1.36 (0.77-2.37)	0.287	1 (0.36-2.73)	0.997
Injection drug users	0.77 (0.36-1.64)	0.496	0.6 (0.18-2.03)	0.409
Subtype				
Non-B (Ref.)				
B	1.18 (0.66-2.1)	0.574	0.98 (0.44-2.2)	0.959
√CD4 counts [a]	1.03 (0.99-1.07)	0.216	0.94 (0.89-0.99)	0.024
Transmission year	1.01 (0.96-1.05)	0.792	1.14 (1.06-1.24)	0.001
Time to ART (years)	1.3 (1.16-1.46)	<0.0001	1.22 (1.05-1.43)	0.01
Chronic RNA VL AUC	2.22 (1.64-3.01)	<0.0001	2.74 (1.77-4.26)	<0.0001

[a] At baseline before treatment

Supplementary Figure 2. Example of potential post-ART transmission in which the infection window of the recipient (red vertical lines, see methods for definitions) overlaps with the ART-start-date (dashed blue line) of the transmitter **(A)**. The black lines depict the viral load measurements of a hypothetical transmitter. In this scenario, the transmission occurred either briefly before or after the initiation of ART. **(B)** The antiretroviral therapy was started before the lower bond of the infection window of the recipient (no overlap), hence in this scenario there is a high degree of certainty that the transmission occurred after ART was initiated by the transmitter.



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Supplementary Material: Chapter II: "*Prescription of Postexposure Prophylaxis for HIV-1 in the Emergency Room: Correct Transmission Risk Assessment Remains Challenging"*

Supplementary Table 1. Demographic data and sexual history divided by PEP decision classification.

	Overall	*Prescribed-and-indicated*	*Prescribed-while-not-indicated*	*Not-prescribed-and-not indicated*	*Not-prescribed-while-indicated*	*P*
n	1051	485	125	294	101	
Age (median [IQR])	32.0 [26.0, 38.0]	33.0 [27.0, 38.0]	31.0 [25.0, 37.0]	30.0 [25.0, 36.0]	32.0 [26.0, 39.0]	<0.001
Sex, female (%)	179 (17.0)	31 (6.4)	46 (36.8)	95 (32.3)	5 (5.0)	<0.001
Swiss nationality (%)	746 (71.0)	349 (72.0)	95 (76.0)	195 (66.3)	76 (75.2)	0.125
MSM (%)	393 (37.4)	278 (57.3)	5 (4.0)	56 (19.0)	48 (47.5)	<0.001
>1 PEP visit (%)	140 (13.3)	86 (17.7)	11 (8.8)	24 (8.2)	12 (11.9)	0.001
Weekend (%)	451 (42.9)	214 (44.1)	48 (38.4)	133 (45.2)	36 (35.6)	0.245
Hours since exposure (median [IQR])	20.0 [10.0, 42.0]	18.0 [9.0, 35.0]	19.0 [11.0, 43.5]	24.0 [10.0, 57.5]	24.0 [10.0, 48.0]	<0.001
Day time (%)						0.143
Midnight-6 AM	181 (17.2)	85 (17.5)	18 (14.4)	52 (17.7)	19 (18.8)	
6 AM - Noon	182 (17.3)	102 (21.0)	25 (20.0)	36 (12.2)	15 (14.9)	
Noon- 6 PM	376 (35.8)	170 (35.1)	44 (35.2)	106 (36.1)	35 (34.7)	
6 PM- Midnight	312 (29.7)	128 (26.4)	38 (30.4)	100 (34.0)	32 (31.7)	
Year (%)						0.423
2007	114 (10.8)	50 (10.3)	14 (11.2)	39 (13.3)	8 (7.9)	
2008	137 (13.0)	63 (13.0)	20 (16.0)	34 (11.6)	15 (14.9)	
2009	168 (16.0)	72 (14.8)	22 (17.6)	54 (18.4)	18 (17.8)	
2010	156 (14.8)	79 (16.3)	19 (15.2)	45 (15.3)	12 (11.9)	
2011	198 (18.8)	105 (21.6)	27 (21.6)	45 (15.3)	19 (18.8)	
2012	149 (14.2)	70 (14.4)	11 (8.8)	41 (13.9)	21 (20.8)	
2013	129 (12.3)	46 (9.5)	12 (9.6)	36 (12.2)	8 (7.9)	
Condom (%)						<0.001
Condom dysfunction	433 (41.2)	208 (42.9)	51 (40.8)	122 (41.5)	48 (47.5)	
Condomless sex	527 (50.1)	263 (54.2)	59 (47.2)	138 (46.9)	51 (50.5)	
With condom	23 (2.2)	1 (0.2)	4 (3.2)	18 (6.1)	0 (0.0)	
Unknown	68 (6.5)	13 (2.7)	11 (8.8)	16 (5.4)	2 (2.0)	
Type of intercourse						
Anal (%)	359 (34.2)	261 (53.8)	9 (7.2)	53 (18.0)	30 (29.7)	<0.001
Vaginal (%)	543 (51.7)	186 (38.4)	106 (84.8)	201 (68.4)	43 (42.6)	<0.001
Oral (%)	157 (14.9)	68 (14.0)	14 (11.2)	43 (14.6)	28 (27.7)	0.002
Only oral (%)	94 (8.9)	34 (7.0)	8 (6.4)	29 (9.9)	21 (20.8)	<0.001
Source partner risk group						
MSM (%)	401 (38.2)	284 (58.6)	6 (4.8)	56 (19.0)	49 (48.5)	<0.001

Sex worker[a] *(%)*	256 (24.4)	177 (36.5)	4 (3.2)	29 (9.9)	44 (43.6)	<0.001
Endemic country (%)	46 (4.4)	32 (6.6)	0 (0.0)	6 (2.0)	7 (6.9)	0.001
Injecting-drug-user (IDU) (%)	11 (1.0)	9 (1.9)	0 (0.0)	1 (0.3)	1 (1.0)	0.137
HIV status of the source partner (%)						<0.001
Negative	175 (16.7)	16 (3.3)	19 (15.2)	136 (46.3)	4 (4.0)	
Positive	131 (12.5)	109 (22.5)	4 (3.2)	12 (4.1)	4 (4.0)	
Unknown	745 (70.9)	360 (74.2)	102 (81.6)	146 (49.7)	93 (92.1)	
Source partner presented the same day (%)	170 (16.2)	21 (4.3)	11 (8.8)	133 (45.2)	4 (4.0)	<0.001
Deciding physician (%)						<0.001
Resident in internal medicine	849 (80.8)	378 (77.9)	104 (83.2)	263 (89.5)	70 (69.3)	
Infectious disease specialist	179 (17.0)	95 (19.6)	20 (16.0)	29 (9.9)	28 (27.7)	
Internal medicine specialist	23 (2.2)	12 (2.5)	1 (0.8)	2 (0.7)	3 (3.0)	

[a] *Sex work in Switzerland is legal and regulated*

[b] *P-values for categorical variables were calculated using chi-square test, for age and hours since exposure a Kruskal–Wallis test was used.*

[c] *Forty-six visits in which the decision could have not been classified due to missing data are included only in the Overall column.*

Supplementary Material: Chapter III "*Mining for pairs: shared clinic visit dates identify steady HIV-positive partnerships"*

Supplementary Text 1

Phylogenetic tree and genetic distances

We used phylogenetic linkage as a key criterion for the validation of the putative steady transmission pairs that were detected using this method. 19,893 partial HIV-1 pol sequences from 10,970 SHCS cohort participants (years range 1989 to 2015) were pooled with 116,408 background, non-Swiss HIV-1 pol sequences from the Los Alamos (http://www.hiv.lanl.gov/) database.The sequences were aligned to a HIV-1 HXB2 [1] reference genome using the MUSCLE [2]. Next, insertions relative to HIV-1 HXB2 and resistance mutations according to Stanford: http://hivdb.stanford.edu/ and International Antiviral Society-USA https://www.iasusa.org/ lists were removed. Finally, a phylogenetic tree was generated using FastTree [3] with the Generalized Time-Reversible (GTR) model. We also calculated tree independent pairwise sequence genetic distances using "TN93"[4] model with the R package “ape” [5]. As a cutoff for evaluation as a serosorting pair we chose a minimal genetic distance of at least 2.5% within a given pair [4, 6], to ensure adequate distance.

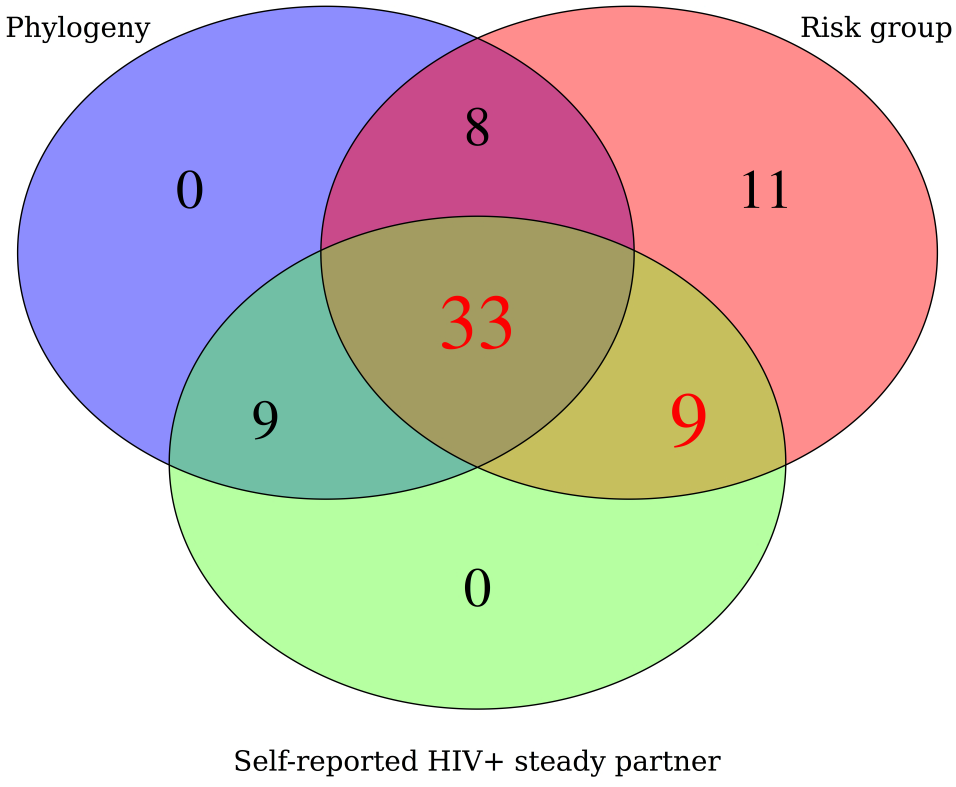
Serosorting pairs characterization

Eight pairs had a high number of effective shared visits (median 13, IQR 8-22), reported an HIV+ steady partner in the SHCS questionnaire, belonged to the same risk group, but were not linked as monophyletic on the phylogeny and showed a high (range 2.6%-13.4%) pairwise distance between the viral sequences. Hence, these pairs are not likely to be transmission pairs - as they have genetically distant viruses- but tenuous serosorting pairs (that require extensive external validation). Four of these pairs were MSM, three heterosexual and one of an HET/IDU risk group. In line with the observation for the steady transmission pairs, two of the MSM pairs were of a mixed ethnicity with White and Latino males, the age gaps being large as well (12 and 19 years). In five pairs the members had a discordant HIV subtype (two pairs with B/01_AE, B/C, F/B and F/Recombinant). All eight pairs reported unprotected sex with a steady partner at least once during the shared visits period, which might be one of the main motivations behind serosorting, but can also facilitate viral superinfection.

Sensitivity analysis of the main simplifying assumption

A core assumption of the binomial model is that visit dates are independently and identically distributed within quarters. One potential source of violation stems from the fact that patients seen at the beginning of one quarter will probably not be seen at the end of the next quarter, and vice versa. Patients seen on the same day or nearby in time for one visit have an increased probability of sharing a date for their next visit (hence constituting a "cohort" within the cohort). The contribution of this effect to the false-positives was tested by narrowing the shuffling window from three months to one months (this value can be modified by the package user) with a corresponding increase of the probability of sharing a single visit (in this case to 1/30, hence increasing the penalty term). Doing so decreased the total yield to 178 pairs with a corresponding decrease of the clearly false-positive to 10% (8/78) (See Supplementary Fig.1 for the Venn diagram) without losing any of the validated 33 transmission pairs. Hence, the user can benefit from adjusting the model to fit the empirical visits scheduling patterns in the specific cohort analyzed.

Supplementary Figure 1. Flowchart and Venn diagram (bottom) demonstrating the validation of 70 putative pairs detected using shared cohort follow-up visit dates. Red numbers indicate putative transmission (n=33) and serosorting pairs (n=9).



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svisits: finding HIV transmission and serosorting pairs using shared clinic visit dates

Alex Marzel, Teja Turk

This package helps to find some stable HIV-infected partnerships in cohort studies based on the assumption that some patients frequently attend the clinical visits together. These pairs are useful for biological and epidemiological research.

Package installation:

```
library(svisits)
set.seed(14051948)
```

The following paragraphs explain step-by-step how the **svisits** package can be used to find stable HIV-infected partnerships. All the steps can be also performed with one function call, which is demonstrated below.

First let us load the database with visit dates in a long format (each row represents a visit of a particular person) and transform it to the appropriate format. The following simulated dataset contains 20 transmission pseudo pairs. The data were simulated based on the visits distribution in the SHCS.

```
data("simulated_data")
# prepare the database
db_dates <- prepare_db(your_database = simulated_data,
                       ids_column = "subject",
                       dates_column = "sim_dates")
head(db_dates)
```

```
##   subject      dates
## 1       1 1993-10-02
## 2       1 1993-12-24
## 3       1 1994-03-16
## 4       1 1994-05-27
## 5       1 1994-08-21
## 6       1 1994-11-06
```

Deconstruct the dates into 3 months periods:

```
# extract month, day and year of each clinic visit:
db_dates <- cbind(db_dates, month.day.year(db_dates$dates))
# deconstruct the dates into 3 months periods
periodMonths <- 3
db_dates <- cbind(db_dates,
                  fupdatePeriod=db_dates$year+round(floor((db_dates$month-1)/periodMonths)
                                                    *periodMonths/12,
                                                    digits=2))
```

Get unadjusted, observed shared visits. It can take some time.

```
unadjusted_observed_pairs <- get_observed_pairs(db_dates)
head(unadjusted_observed_pairs)
```

```
## allPairs
## 1000_1163 1000_1168 1000_1258 1000_1310 1000_1347 1000_1388
##         1         1         1         1         1         1
```

Descriptive statistics of shared visits for pairs that shared at least a single visit:

```
summary(as.numeric(unadjusted_observed_pairs))
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   1.000   1.000   1.000   1.321   1.000  24.000
```

Prepare the ids:

```
ids_table <- table(db_dates$subject)
ids <- data.table(ids = as.character(names(ids_table)),
                  N_visits = as.character(as.numeric(ids_table)))
setkey(ids, "ids")
```

Chances that two unrelated cohort members share a visit are increasing with the overall number of visits of each member of the pair. To account for this we have to adjust the number of shared visits S within each pair. This will return the adjusted number of shared visits S' for the observed, unshuffled data

$$S' = S - \frac{\log\left[\binom{\min(T_a,T_b)}{S}\right]}{\log\left[75\right]},$$

where T_a and T_b denote the number of visits of each member of the pair. The adjusted number of visits is under the column "Corrected":

```
adjusted_observed <- adjust_visits(unadjusted_pairs = unadjusted_observed_pairs,
                                   ids = ids,
                                   prob = 1/75)
head(adjusted_observed)
```

```
##   allPairs Freq id_1 id_2 N_visits.x N_visits.y Corrected
## 1     1_10    1    1   10         46         68 0.1132248
## 2     4_10    1    4   10         39         68 0.1514599
## 3     8_10    1    8   10         10         68 0.4666841
## 4     9_10    1    9   10         12         68 0.4244555
## 5 161_1000    1  161 1000         17         11 0.4446087
## 6 176_1000    1  176 1000         11         11 0.4446087
```

Threshold for the number of shared visits

Shuffling

Due to the multiple comparisons problem and strongly simplifying assumptions about the uniform distribution of visits, the corrected number of shared visits is not an optimal test statistic. Instead, the visits are first shuffled within each quarter (such that the original distribution of the number of visits per individual is preserved) and the number of randomly collided shared visits per pair is counted and penalized using "adjust_visits". In other words, the observed visit dates from a given quarter are randomly re-assigned between the patients that attended the clinic during this quarter.

Define the number of desired shuffling simulations (ideally > 100, but it will take some time) and run. It will take some time to finish.

```
n_shuffl <- 10
Shuffling_simulation_output <- replicate(n_shuffl,
                                         Shuffling_simulation(db_dates,
                                                              ids = ids,
                                                              adjusted_observed),
                                         simplify=F)
```

```r
# inspect the false-positive thresholds
head(Shuffling_simulation_output)
```

```
## [[1]]
## [1] 0.9394822 0.7810526 0.3095238 0.0000000 0.0000000 0.0000000 0.0000000
## [8] 0.0000000
##
## [[2]]
## [1] 0.9434465 0.7515789 0.2857143 0.0000000 0.0000000 0.0000000 0.0000000
## [8] 0.0000000
##
## [[3]]
## [1] 0.9418979 0.6905263 0.1904762 0.0000000 0.0000000 0.0000000 0.0000000
## [8] 0.0000000
##
## [[4]]
## [1] 0.9389866 0.6673684 0.2619048 0.0000000 0.0000000 0.0000000 0.0000000
## [8] 0.0000000
##
## [[5]]
## [1] 0.9485258 0.6547368 0.1428571 0.0000000 0.0000000 0.0000000 0.0000000
## [8] 0.0000000
##
## [[6]]
## [1] 0.92758920 0.65263158 0.30952381 0.04761905 0.00000000 0.00000000
## [7] 0.00000000 0.00000000
```

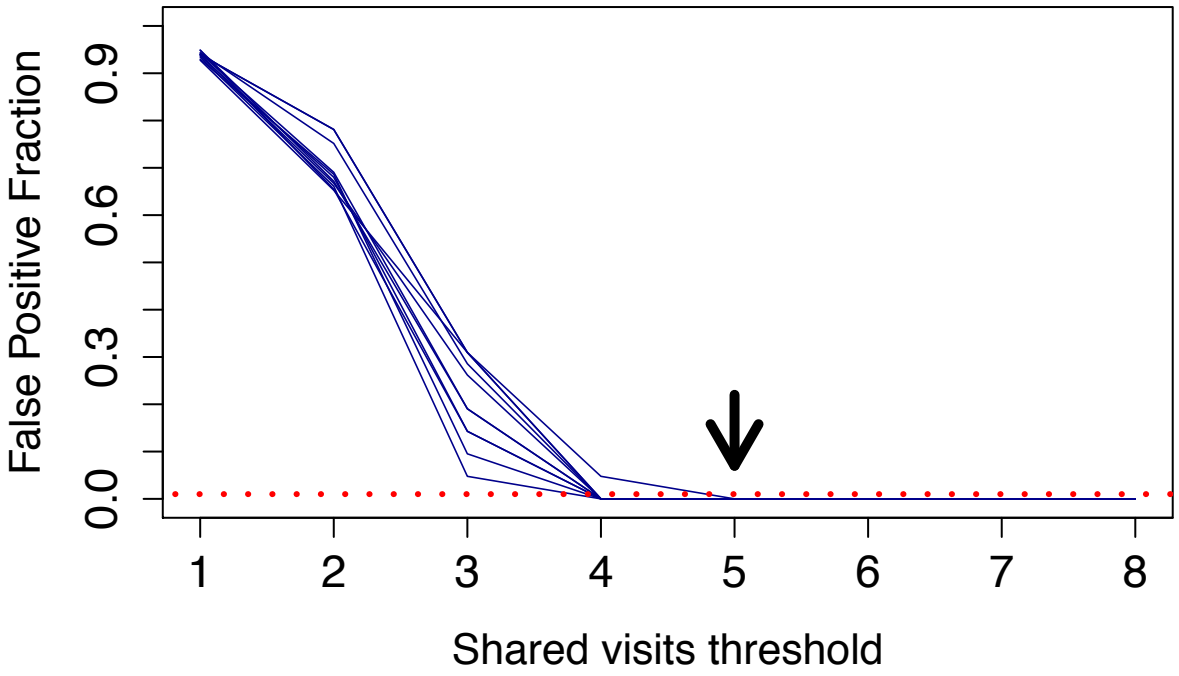
Extract the False-Positive thresholds:

```r
# Thresholds
for_thresholds <- do.call("rbind", Shuffling_simulation_output)

# Take max FP value for each position from all the simulations.
# One can also take mean or median insted of maximum
for_thresholds_max <- apply(X = for_thresholds,
                            MARGIN = 2,
                            max,na.rm = TRUE)

# find lowest threshold below alpha=0.01
Thresholds_lowest <- match(for_thresholds_max[for_thresholds_max<0.01][1],
                           for_thresholds_max)
```

Plot the simulations and the False-Positive threshold (indicated by an arrow):

```r
max_threshold <- length(Shuffling_simulation_output[[1]])
plot(x=1:max_threshold,
     y=unlist(Shuffling_simulation_output[[1]]),
     xlab="Shared visits threshold", ylab="False Positive Fraction",
     type="l", col="darkblue", yaxp=c(0.0,1,10), lwd=0.8, main="",
     xaxp=c(1,12,11), cex.lab=1.3, cex.axis=1.4, cex.main=1.3, ylim=c(0,1))
arrows(x0 = Thresholds_lowest, x1=Thresholds_lowest,
       y0=0.22, y1=0.07, cex=3, lwd=5)
invisible(lapply(X=1:n_shuffl,
                 function(X) {
                   lines(x=1:max_threshold, y=unlist(Shuffling_simulation_output[[X]]),
```

```
                        col="darkblue", lwd=0.8 )
                  }))
lines(c(-100,100), 0.01*c(1,1), lty=3, col="red", lwd=3)
```




```
Thresholds_lowest
```

```
## [1] 5
```

```
# This is the cutoff for the number of adjusted visits
```

Extract the pairs above the threshold:

```
select_shuffled <- adjusted_observed[which(adjusted_observed$Corrected>Thresholds_lowest),]

# We found the 20 pairs
length(select_shuffled[,1])
```

```
## [1] 20
```

Alternative (and much faster approach): Bonferroni correction

Predict the probabilities and than adjust for multiple testing using Bonferroni:

```
# alpha 0.01, one false-positive pair
Bonferroni_m_output <- Bonferroni_m(unadjusted_observed_pairs,
                                    ids = ids,
                                    prob = 1/75,
                                    alpha =0.01)
length(Bonferroni_m_output[,1])
```

```
## [1] 21
```

```
# alpha 0.001, only 20 true pairs
Bonferroni_m_output <- Bonferroni_m(unadjusted_observed_pairs,
                                    ids = ids,
```

```
                                    prob = 1/75,
                                    alpha =0.001)
length(Bonferroni_m_output[,1])
```

```
## [1] 20
```

Summary: one-step-approach

The above pairs can be alternatively found with one function call:

```
library(svisits)
data("simulated_data")
set.seed(14051948)

# with shuffling and Bonferroni for alpha=0.01
pairs <- find_pairs(simulated_data,
                    ids_column = "subject",
                    dates_column = "sim_dates",
                    shuffling = TRUE,
                    n_shuffl = 10)
head(pairs$Shuffled_pairs)
```

```
##         allPairs Freq id_1 id_2 N_visits.x N_visits.y Corrected
## 62746   328_1453   22  328 1453         38         44  16.48171
## 65536  1173_1468   24 1173 1468         70         77  14.10959
## 85836   482_1587   21  482 1587         77         66  11.96045
## 101885 1504_1696   17 1504 1696         44         37  11.55935
## 141792   72_1898   16   72 1898         33         39  11.16443
## 142396  793_1900   18  793 1900         44         37  12.53495
```

```
# show similarity
identical(select_shuffled, pairs$Shuffled_pairs)
```

```
## [1] TRUE
```

```
# with only Bonferroni for alpha=0.001
pairs_Bonferroni <- find_pairs(simulated_data,
                    ids_column = "subject",
                    dates_column = "sim_dates",
                    alpha = 0.001,
                    shuffling = FALSE)
head(pairs_Bonferroni$Bonferroni_pairs)
```

```
##         allPairs Freq id_1 id_2 N_visits.x N_visits.y Prob_for_Bonferr
## 62746   328_1453   22  328 1453         38         44     1.005805e-31
## 65536  1173_1468   24 1173 1468         70         77     1.885820e-27
## 85836   482_1587   21  482 1587         77         66     2.046927e-23
## 101885 1504_1696   17 1504 1696         44         37     1.617750e-22
## 141792   72_1898   16   72 1898         33         39     9.266487e-22
## 142396  793_1900   18  793 1900         44         37     2.429054e-24
##            BP      ltp
## 62746   62746 30.99749
## 65536   65536 26.72450
## 85836   85836 22.68890
## 101885 101885 21.79109
```

```
## 141792 141792 21.03308
## 142396 142396 23.61456
```

```
# show similarity
identical(Bonferroni_m_output, pairs_Bonferroni$Bonferroni_pairs)
```

```
## [1] TRUE
```

Supplementary Material: Chapter IV *"The cumulative impact of harm reduction on the Swiss HIV epidemic: cohort study, mathematical model and phylogenetic analysis"*

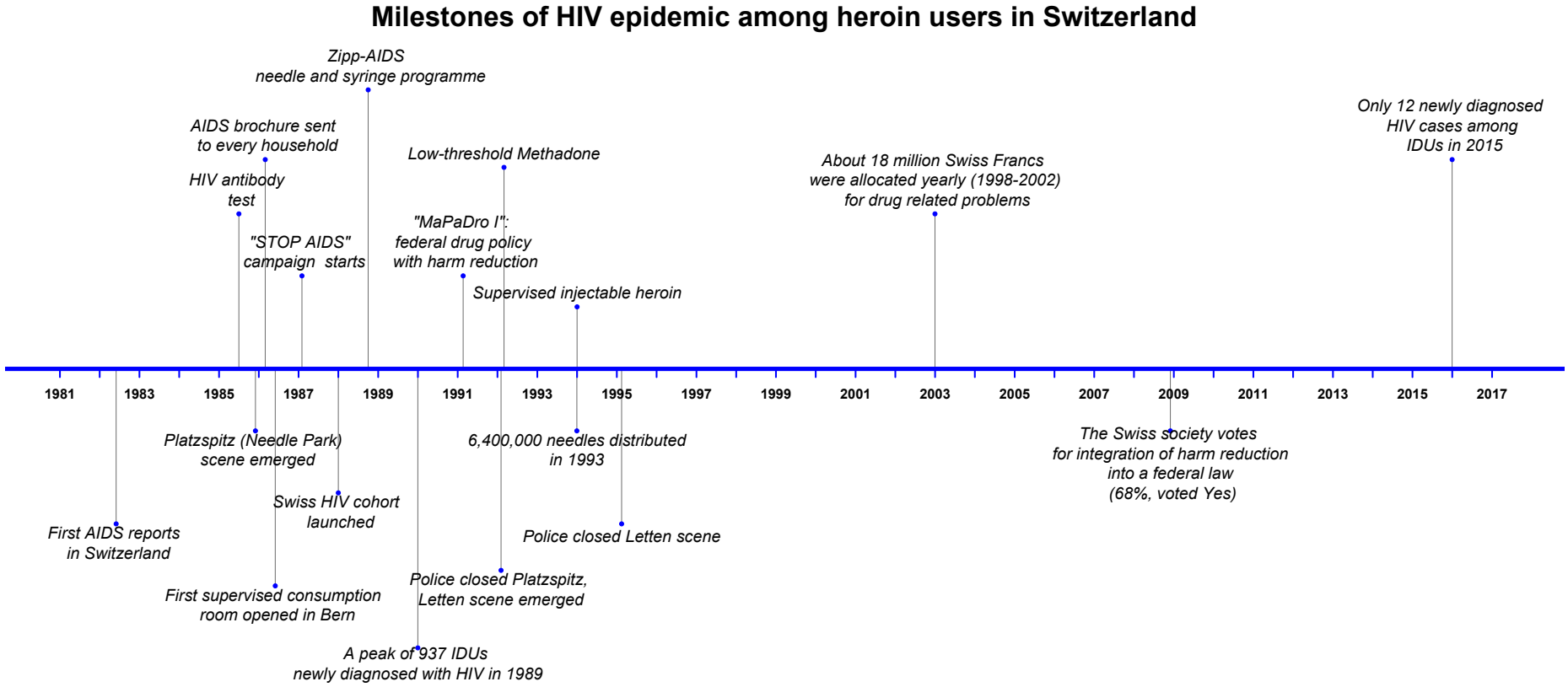
SUPPLEMENTARY MATERIAL

The cumulative impact of harm reduction on the Swiss HIV epidemic: cohort study, mathematical model and phylogenetic analysis

Supplementary Table 1. IDUs in the Swiss HIV Cohort Study, 1980-2016, by diagnosis period.

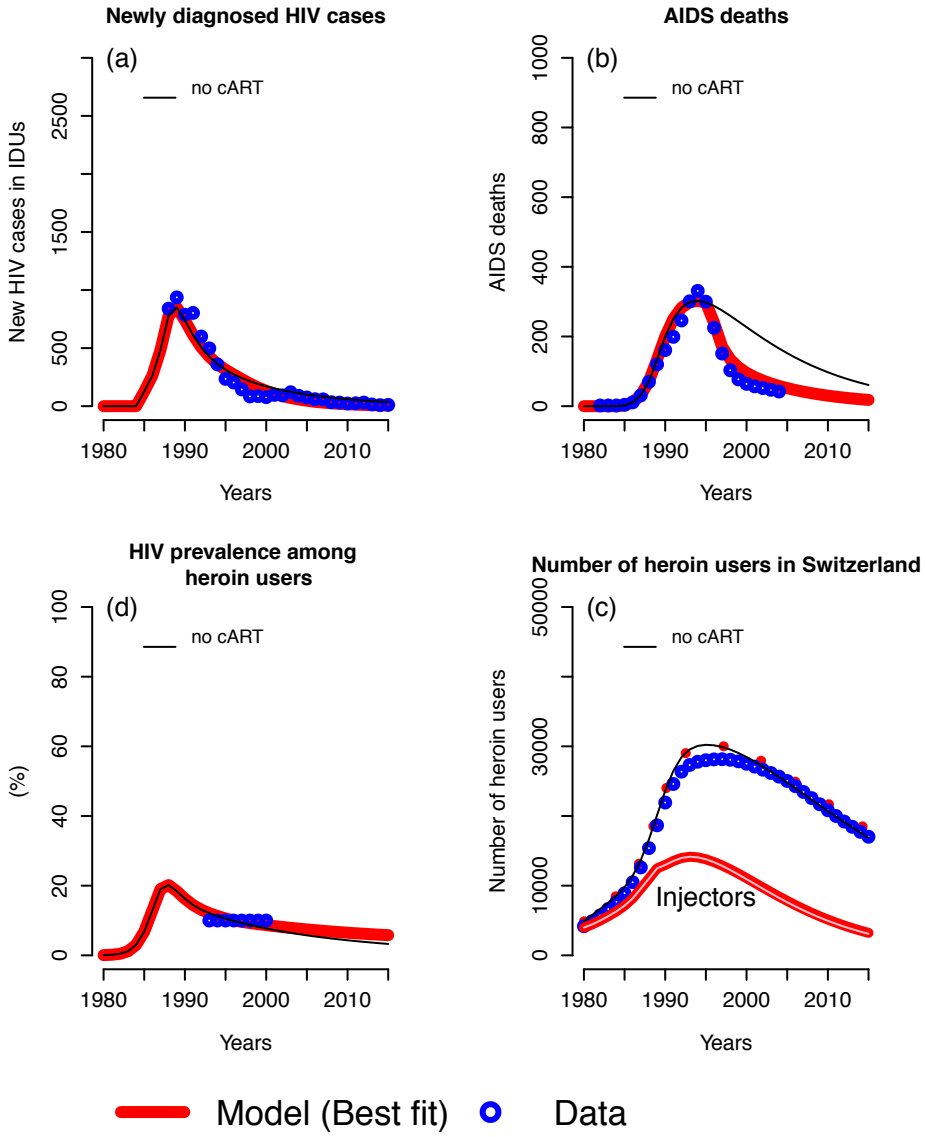
		Overall	[1980,1990)	[1990,2000)	[2000,2010)	[2010,2016]	P for trend	Phylogeny
n		4806	2999	1344	394	69	0.044	2399
Sex	Male	3157 (65.7)	1932 (64.4)	896 (66.7)	278 (70.6)	51 (73.9)	0.005	1548 (64.5)
	Female	1649 (34.3)	1067 (35.6)	448 (33.3)	116 (29.4)	18 (26.1)		851 (35.5)
Age at diagnosis (median [IQR])		27.0 [23.0, 31.0]	25.0 [23.0, 28.0]	29.0 [25.0, 33.0]	32.0 [28.0, 38.0]	36.0 [31.0, 47.0]	0.002	27.0 [23.0, 31.0]
University education	Yes	45 (0.9)	22 (0.7)	12 (0.9)	6 (1.5)	5 (7.2)	0.163	22 (0.9)
	No	4761 (99.1)	2977 (99.3)	1332 (99.1)	388 (98.5)	64 (92.8)		2377 (99.1)
Untreated CD4 (median [IQR])		379.0 [190.0, 600.0]	370.0 [185.5, 600.0]	378.0 [186.0, 600.0]	406.5 [238.8, 632.5]	366.0 [182.0, 535.5]	0.883	420.0 [240.0, 640.0]
Years to cART (median [IQR])		9.2 [3.6, 12.6]	12.2 [10.6, 15.2]	5.1 [2.4, 7.9]	1.6 [0.2, 3.5]	0.2 [0.1, 0.8]	0.048	9.1 [3.8, 12.6]
All time AIDS	Yes	2010 (41.8)	1441 (48.0)	485 (36.1)	73 (18.5)	11 (15.9)	0.032	837 (34.9)
	No	2796 (58.2)	1558 (52.0)	859 (63.9)	321 (81.5)	58 (84.1)		1562 (65.1)
HBV	Ever positive	2312 (78.3)	1249 (83.9)	807 (79.5)	217 (56.7)	39 (58.2)	0.087	1742 (78.2)
	Negative	642 (21.7)	240 (16.1)	208 (20.5)	166 (43.3)	28 (41.8)		485 (21.8)
HBV missing	Yes	1852 (38.5)	1510 (50.4)	329 (24.5)	11 (2.8)	2 (2.9)	0.062	172 (7.2)
	No	2954 (61.5)	1489 (49.6)	1015 (75.5)	383 (97.2)	67 (97.1)		2227 (92.8)
HCV	Ever positive	2728 (94.6)	1364 (96.2)	948 (94.0)	367 (94.3)	49 (73.1)	0.184	2117 (94.8)
	Negative	155 (5.4)	54 (3.8)	61 (6.0)	22 (5.7)	18 (26.9)		117 (5.2)
HCV missing	Yes	1923 (40.0)	1581 (52.7)	335 (24.9)	5 (1.3)	2 (2.9)	0.071	165 (6.9)
	No	2883 (60.0)	1418 (47.3)	1009 (75.1)	389 (98.7)	67 (97.1)		2234 (93.1)

Supplementary Figure 1. Selected milestones of HIV epidemic among heroin users in Switzerland, 1981-2016. Sources: (1)(2)(3)(4)(5)(6).

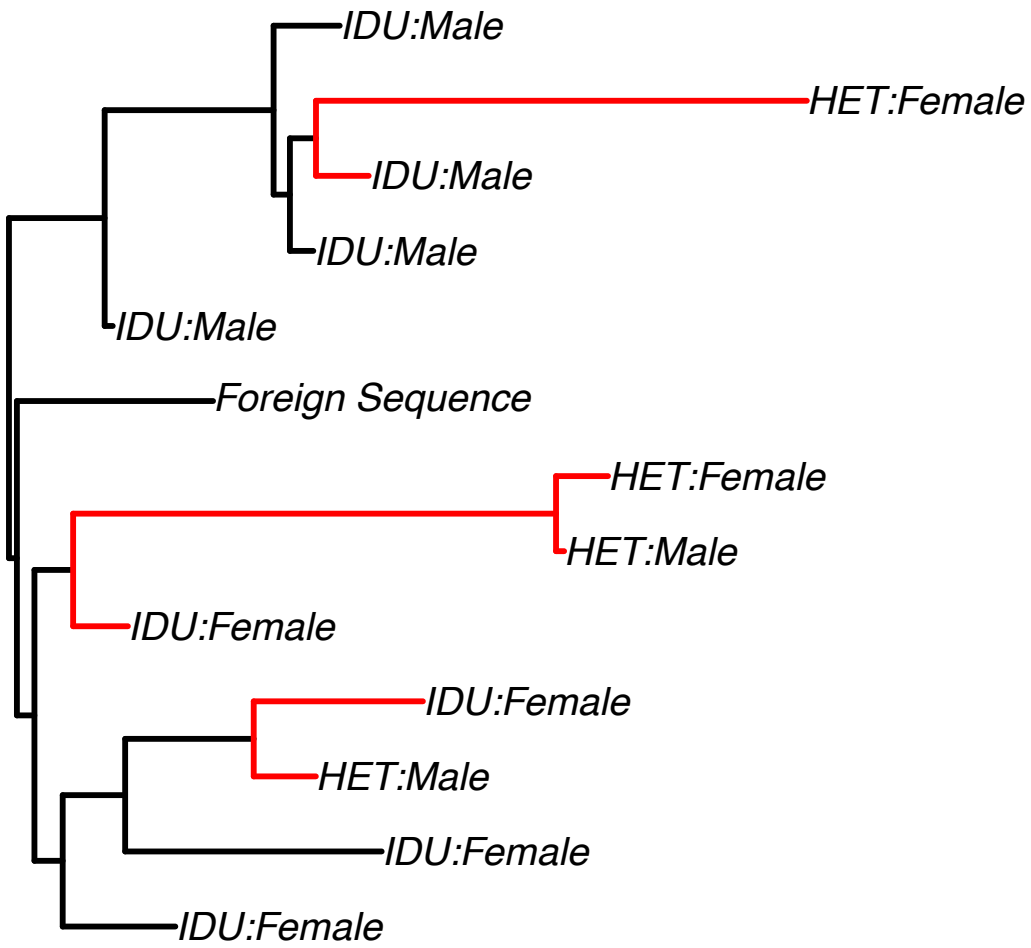


Supplementary Figure 2. Supervised drug consumption room, Oerlikon, Zürich.

Supplementary Figure 3. Counterfactual scenario with no cART introduction.



Supplementary Figure 4. Subsection of the phylogenetic tree that demonstrates three introduction events into the heterosexual population (red). Multiple sequences from the same patients were dropped for clarity, hence each tip represents a different patient. Abbreviations: HET: heterosexual, IDU: Injecting-Drug-User.



Supplementary Table 2. Model parameters and assumptions. Model parameters were obtained either by model fitting to the reported number of HIV cases and AIDS deaths, from the Swiss HIV Cohort Study or were approximated based on available literature preferably from historically comparable settings. Sensitivity analysis was performed by varying key model parameters (see below).

Parameter	Description and assumptions	Value (range)	Source
b(t)	Estimated annual number of new problematic heroin users in Switzerland	Vector of 36 values 1980 to 2015: 1153, 1200, 1245, 1311, 1456, 1755, 2278, 3046, 3947, 4683, 4892, 4468, 3687, 2929, 2379, 2028, 1793, 1603, 1431, 1277, 1143, 1024, 917, 821, 736, 659, 590, 529, 474, 424, 380, 341, 305, 273, 245, 219 Years 2010 to 2015 were extrapolated using Generalized additive model (GAM) with integrated smoothness estimation from the R "mgcv" package (version 1.8-17)	Incidence of heroin use from (7) was extrapolated until 2015, Data for the Swiss population size was obtained from the Swiss Federal Statistical Office (8)
f_iv	Fraction of heroin users that start their heroin consumption via intravenous route	0.27	(9)

v(t)	Rate of transition from non-intravenous heroin use to injecting	$$\mathrm{v}(t) = \begin{cases} \dfrac{1}{2.08\ yr}, & t < 1990 \\ (\dfrac{1}{2.08\ yr})/2, & t \geq 1990 \end{cases}$$ We make this transition two times slower after 1990 to account for the assumption that due to the increasing awareness of the HIV epidemic and the high overdose rate many non-injectors might be reluctant to switch to the injecting drug administration mode.	(10) (11)
fr_iv	Transition rate from being an active intravenous user to either non-intravenous mode (smoking, snorting or methadone), or to a supervised injectable heroin.	Although the exact rate is unknown, data from Europe shows that this transition was not uncommon. Griffiths et al. (1994) showed that, 16% of current chasers were previously injectors (11). de la Fuente (1994) showed that fear of contracting HIV played an important role in this transition (12). We set this rate at $\frac{1}{6.25\ yr}$ and only allow this transition from the harm reduction covered compartment.	(11) (12)
m_n	Death rate and emigration, for non-intravenous users, not on harm reduction	$$\frac{1}{43\ yr}$$ Based on life expectancy of 75 (in 1980) and a mean age of heroin use initiation of 20, plus 0.5% emigration * the curve from (7) that describes the estimated annual number of heroin users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	(13)

m_n_h	Death rate and emigration, for non-intravenous users, on harm reduction	$\frac{1}{48\ yr}$ Based on life expectancy of 75 (in 1980) and a mean age of heroin use initiation of 20, plus 0.5% emigration We assume 10% lower rate on harm reduction * the curve from (7) that describes the estimated annual number of heroin users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	(13)
m_s_i	Death and emigration for susceptible, and infected in the recent, chronic or treated non-AIDS stages, intravenous users, not on harm reduction	$\frac{1}{24\ yr}$ Sordo *et al.* reported pooled all-cause mortality of 27.7^{-1y} out of methadone, plus 0.5% emigration * the curve from (7) that describes the estimated annual number of heroin users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	(14)
m_s_i_h	Death and emigration for susceptible, recent stage, chronic or treated non-AIDS stage, covered by any harm reduction	$\frac{1}{48\ yr}$ Sordo *et al.* reported pooled all-cause mortality rates of 88^{-1y} on methadone. However, this is a recent estimate. Since it is implausible that the death rate of non-injectors was faster than that of the active injectors (m_n_h), we fix the rate 39^{-1y} * the curve from (7) that describes the estimated annual number of heroin	(14)

		users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	
m_a	Death rate from AIDS for untreated patients, we assume the same rate for people on and off harm reduction	$\frac{1}{1.6\ yr}$	(15)
m_at	AIDS death rate on HAART. Assumption: for some patients in the AIDS stage, treatment might be administrated too late.	$\frac{1}{9.09\ yr}$	Assumed, based on (16)

w	Successful withdrawal rate not on any harm reduction ("cold turkey"). Here withdrawal means complete cessation of opioid use, including methadone and non-injection	$\frac{1}{50\ yr}$ Nordt and Stohler estimated a cessation rate of 4%, however this estimate included emigration and death and was based on a methadone registry, we assume 2% unassisted withdrawal * the curve from (7) that describes the estimated annual number of heroin users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	(7)
w_h	Successful withdrawal rate on harm reduction Here withdrawal means complete cessation of opioid use, including methadone and non-injection	$\frac{1}{33\ yr}$ Nordt and Stohler estimated a cessation rate of 4%, however this estimate included emigration and death and was based on a methadone registry. The cessation rate is higher on harm reduction since oftentimes maintenance medication serves as a gateway for cessation (17). * the curve from (7) that describes the estimated annual number of heroin users in Switzerland, serves as an external control for the combined impact of all the deaths, emigration and cessation rates (Figure 3 in the manuscript)	(7,17)

r_c	Transition rate from recent to chronic HIV infection stage	$\frac{1}{0.5\ yr}$	Definition of recent infection
c_a	Transition rate from chronic to AIDS HIV infection stage	$\frac{1}{8\ yr}$	(18)
a(t)	HIV diagnosis rate, not on harm reduction	$\mathrm{a}(t) = \begin{cases} 0, & t < 1985 \\ \frac{1}{3.48\ yr}, & t \geq 1985 \end{cases}$ Time to diagnosis was estimated based on the SHCS data, by taking the time interval between the HIV positive date and the last known HIV negative date (and not the midpoint as in (19)). We assume that most SHCS patients were covered by harm reduction, and that that the diagnosis rate was 50% slower in compartments not covered by harm reduction	SHCS

a_h(t)	HIV diagnosis rate, covered by harm reduction	$$\text{a\_h}(t) = \begin{cases} 0, & t < 1985 \\ \frac{1}{1.74\ yr}, & t \geq 1985 \end{cases}$$ Time to diagnosis was estimated based on the SHCS data, by taking the time interval between the HIV positive date and the last known HIV negative date (and not the midpoint as in (19)). We assume that all SHCS patients were covered by harm reduction, and that that the diagnosis rate was 50% times slower in compartments not covered by harm reduction	SHCS
g_R(t)	HAART initiation rate, For IDU not on harm reduction, that are in a recent infection stage	$$\text{g\_R}(t) = \begin{cases} 0, & t < 1996 \\ 0.131 + 0.038 * (\text{t} - 17), & t \geq 1996 \end{cases}$$ Estimated based on SHCS data, using time to event analysis with IDUs with a CD4 value of >500 close to diagnosis as a proxy for the recent infection phase (Supplementary Table 3) Since the clinical practice gradually transitioned toward immediate treatment (20), the rate increases linearly with a slope 0.038 per year which will enforce immediate treatment in 2015. Uhlmann et al. (21) reported a 62% increase in ART initiation among people on methadone as compared to non-methadone. Hence, we assumed a 62% slower rate for the parallel non-harm reduction compartment. This does not apply to the AIDS stage, in which people will be treated regardless of their harm reduction coverage.	SHCS

g_R_h (t)	HAART initiation rate, For IDU on harm reduction, that are in a recent infection stage	$\text{g\_R\_h}(t) = \begin{cases} 0, & t < 1996 \\ 0.131 + 0.038 * (\text{t} - 17), & t \geq 1996 \end{cases}$ Estimated based on SHCS data, using time to event analysis with IDUs with a CD4 value of >500 close to diagnosis as a proxy for the recent infection phase (Supplementary Table 3) Since the clinical practice gradually transitioned toward immediate treatment (20) , the rate increases linearly with a slope 0.038 per year which will enforce immediate treatment in 2015. We assume that all the SHCS patients were covered by harm reduction, since it would be unlikely to participate in the cohort while bypassing all the harm reduction measures. Uhlmann et al. (21) reported a 62% increase in ART initiation among people on methadone as compared to non-methadone. Hence, we assumed a 62% slower rate for the parallel non-harm reduction compartment. This does not apply to the AIDS stage, in which people will be treated regardless of their harm reduction coverage.	SHCS
g_C(t)	HAART initiation rate, For IDU not on harm reduction, that are in a chronic infection stage	$\text{g\_C}(t) = \begin{cases} 0, & t < 1996 \\ 0.24 + 0.071 * (\text{t} - 17), & t \geq 1996 \end{cases}$ Estimated based on SHCS data, using time to event analysis with IDUs with a CD4 values between 200 and 500, close to diagnosis, as a proxy for the chronic infection phase (Supplementary Table 3) Since the clinical practice gradually transitioned toward immediate treatment (20), the rate increases linearly with a slope 0.071 per year which will enforce	SHCS

		immediate treatment in 2015. We assume that all the SHCS patients were covered by harm reduction, since it would be unlikely to participate in the cohort while bypassing all the harm reduction measures. Uhlmann et al. (21) reported a 62% increase in ART initiation among people on methadone as compared to non-methadone. Hence, we assumed a 62% slower rate for the parallel non-harm reduction compartment. This does not apply to the AIDS stage, in which people will be treated regardless of their harm reduction coverage.	
g_C_h (t)	HAART initiation rate, For IDU on harm reduction, that are in a chronic infection stage	$\text{g\_C\_h}\ (t) = \begin{cases} 0, & t < 1996 \\ 0.24\ +\ 0.071\ *\ (\text{t} - 17), & t \geq 1996 \end{cases}$ Estimated based on SHCS data, using time to event analysis with IDUs with a CD4 values between 200 and 500, close to diagnosis, as a proxy for the chronic infection phase (Supplementary Table 3) Since the clinical practice gradually transitioned toward immediate treatment (20), the rate increases linearly with a slope 0.071 per year which will enforce immediate treatment in 2015. We assume that all the SHCS patients were covered by harm reduction, since it would be unlikely to participate in the cohort while bypassing all the harm reduction measures. Uhlmann et al. (21) reported a 62% increase in ART initiation among people on methadone as compared to non-methadone. Hence, we assumed a 62% slower rate for the parallel non-harm reduction compartment. This does not apply to the AIDS stage, in which people will be treated regardless of their	SHCS

		harm reduction coverage.	
g_A(t)	HAART initiation rate, For IDU not on harm reduction, that are in AIDS stage	$\text{g\_A}(t) = \begin{cases} 0, & t < 1996 \\ 0.55 + 0.138 * (\text{t} - 17), & t \geq 1996 \end{cases}$ Estimated based on SHCS data, using time to event analysis with IDUs with CD4 values below 200, close to diagnosis, as a proxy for the AIDS infection phase (Supplementary Table 3) Time to HAART was calculated as the time interval between the first CD4 value <200 (after 1996) and HAART initiation Since the clinical practice gradually transitioned toward immediate treatment (20), the rate increases linearly with a slope 0.138 per year which will enforce immediate treatment in 2015. We assume that all the SHCS patients were covered by harm reduction, since it would be unlikely to participate in the cohort while bypassing all the harm reduction measures.	SHCS

g_A_h(t)	HAART initiation rate, For IDU on harm reduction, that are in AIDS stage	$\text{g\_A\_h}(t) = \begin{cases} 0, & t < 1996 \\ 0.55 + 0.138 * (\text{t} - 17), & t \geq 1996 \end{cases}$ Estimated based on SHCS data, using time to event analysis with IDUs with CD4 values below 200, close to diagnosis, as a proxy for the AIDS infection phase (Supplementary Table 3) Since the clinical practice gradually switched toward immediate treatment (20), the rate increases linearly with a slope 0.138 per year which will enforce immediate treatment in 2015. We assume that all the SHCS patients were covered by harm reduction, since it would be unlikely to participate in the cohort while bypassing all the harm reduction measures.	SHCS
r	HAART interruption and drug resistance, not on harm reduction	$\frac{1}{4.76\ yr}$ Estimated using time-to-event analysis of the IDUs in the SHCS that initiated HAART. The time variable was calculated from HAART initiation date until the first two consecutive HIV RNA measurements above 500 copies/mL. For individuals without interruption/resistance the date was censored at the last available follow-up. The estimated rate was $\frac{1}{7.15\ yr}$ Since Palepu *et al.* found that methadone maintenance therapy (MMT) was positively associated with adherence (AOR 1.52; 95% CI 1.16-2.00), the rate for the not-covered by harm reduction compartment was increased by	SHCS (22)(21)(15)

		52%.	
r_h	HAART interruption and drug resistance, on harm reduction	Estimated using time-to-event analysis of the IDUs in the SHCS that initiated HAART. The time variable was calculated from HAART initiation date until the first two consecutive HIV RNA measurements above 500 copies/mL. For individuals without interruption/resistance the date was censored at the last available follow-up. The estimated rate was $\frac{1}{7.15\ yr}$	(21)(15) SHCS (23,24)

h(t)	Annual rate of recruitment to any harm reduction	$h(t) = \begin{cases} \text{estimate (see below) fit: } \frac{1}{11.7\ yr}, & t < \text{h\_t\_start} \\ \text{model fit: } 1.44\ (95\%\ C.I\ 1.16 - 1.71)\ , & t \geq \text{h\_t\_start} \end{cases}$ Before 1988, only restricted methadone was available, After 1988 needle exchange and extended methadone were added, later in 1994 a supervised injectable heroin as well. The rate before 1988 ($\frac{1}{11.7\ yr}$), was estimated by fitting the following ODE system to the reported restricted methadone data (1980-1987) $\frac{dI}{dt} = b(t) + o \cdot M - h \cdot I - \mu \cdot I$ $\frac{dM}{dt} = h \cdot I - o \cdot M - \mu \cdot M$ Where the compartment I represents IDU not on methadone, the compartment M IDU on methadone. b(t) new heroin users, o methadone drop-out ($\frac{1}{3.76\ yr}$), h methadone recruitment (model fit) and μ death, emigration and cessation rates ($\frac{1}{25\ yr}$). with initial conditions: I = 3857, M = 733. See Supplementary Figure 5.	For the number of patients on restricted Methadone (25) After h_t_start: model fit

h_hivp(t)	Annual rate of recruitment to harm reduction, for diagnosed HIV positive individuals	2 * h(t) Gervasoni et al. show that HIV positive individuals have two times higher odds for being on methadone as compared to HIV negative.	(26)
o	Annual rate of loss to harm reduction package	Hissoud et al (27) showed that the proportion of remaining on methadone treatment was 69% at 1 year and 45% at 3 years, hence, we use the drop-out rate: $\frac{1}{3.76\ yr}$	(27)
h_t_start	Year in which the intensive harm reduction package was introduced and started to take an effect	1988 Start of the “Zipp-Aids” Needle and syringe exchange interventions	(25) (5,6)

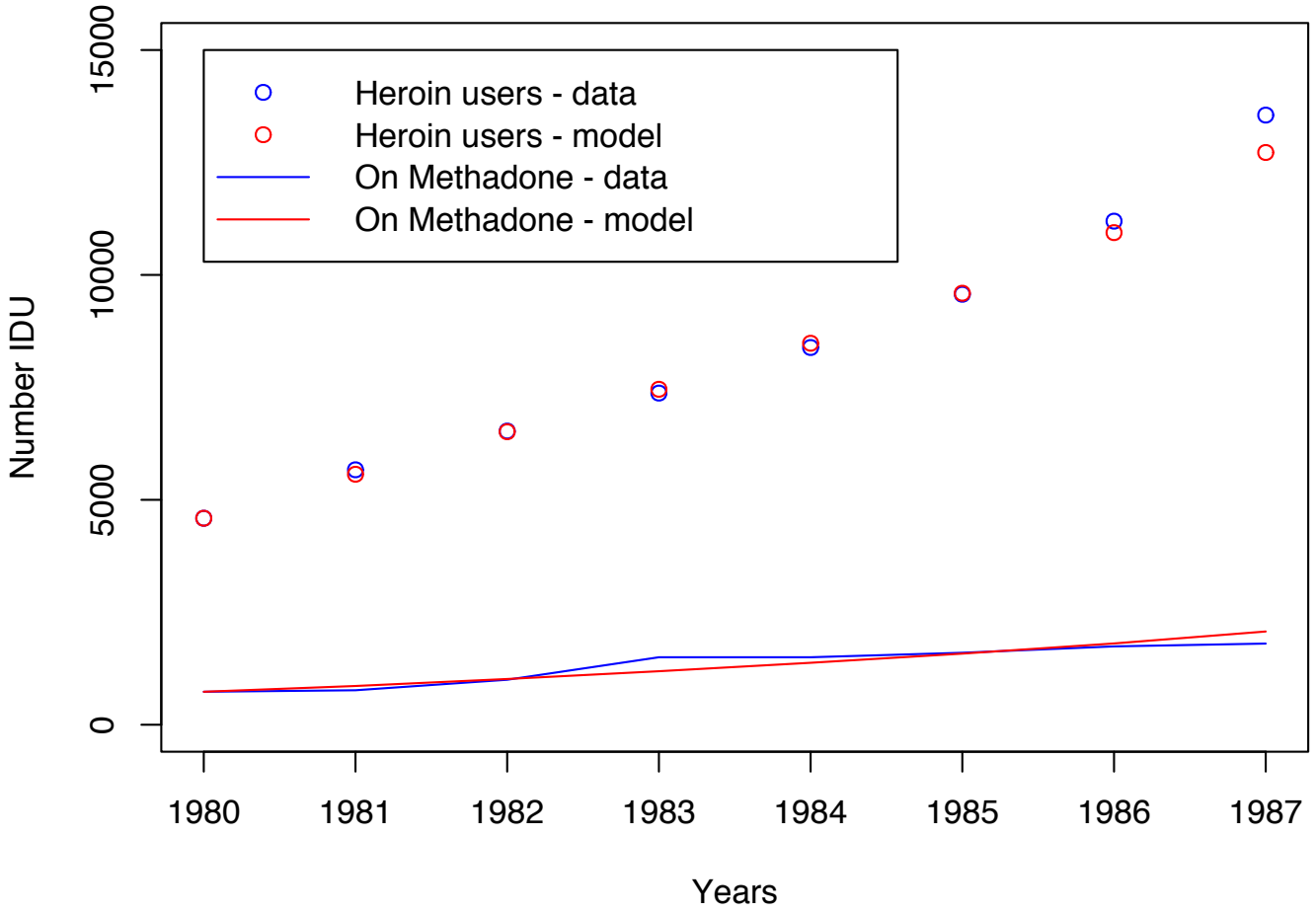
d_s	Fraction of HIV diagnosed IDU that will still share injection paraphernalia	0.32 Wilson et al (28) showed that IDU that were aware of their HIV positive status had a three times lower odds to share needles as compared to HIV negative (odds ratio 0.32, 95% C.I 0.11-0.96). No reduction in risk behavior was found in HIV positive individuals that were unaware of their infection (odds ratio 1.14 95% C.I 0.58-2.2) On harm reduction: we assume 50% lower fraction	(28)
π	A multiplier that represents the fraction of the initial non-harm reduction transmission coefficient that will remain after reduction in risk behavior due to overall increase in HIV awareness.	0.4 van Ameijden (29) showed based on data from the Netherlands, that borrowing needles/syringes has decreased from 51% in 1986 to 20% in 1992. This reduction was similar for people that attended methadone and needle exchange and for people that did not. We are not aware of this kind of evidence for Switzerland, nevertheless we set the value on 40% to obtain the adjusted effect of harm reduction.	(29)
$\beta_{(t)}$	Transmission coefficient, not on harm reduction	Dynamic rate $\beta_{(t)} = \begin{cases} \beta_0, & t \leq 1987 \\ K - (K - \beta_0) \cdot e^{(-\beta_r \cdot (t-8))}, & t > 1987 \end{cases}$ β_0 = $transmission$ coefficient $before$ 1987, $fitted$ (1.25, 95% C.I 1.24-1.26), see below β_r= model fit 0.6 (95% 0.45-0.75) $K = \beta_0 \cdot \pi$ = the lower bound of the initial β_0 , after adjusting for reduction of risk behavior that is independent of harm reduction,	Model fit

		just due to HIV awareness. The interpretation of β_0: Using the common notation for the force of infection of sexually transmitted infections: $$\frac{\beta_p \cdot c \cdot I}{N}$$ β_0 represents the per partnership transmission probability (β_p) that combines both injecting paraphernalia sharing partnerships and sexual partnerships with other active injectors, multiplied by the combined sharing and sexual IDU partners change rate (c). Hence, $\beta_0 = \beta_p \cdot c$, and since both quantities are unknown, and mostly likely also changed with time, we fitted them as one.	
$\beta_{\mathrm{h}}(t)$	Transmission coefficient, on harm reduction	Dynamic rate, represented as a step-function. $$\beta_{\mathrm{h}}(t) = \begin{cases} \varphi \cdot \beta_0, & t \le h\_t\_start \\ (\varphi^2) \cdot \beta_0, & t > h\_t\_start \end{cases}$$ φ is the fraction of the initial non-harm reduction transmission rate (β_0) that is remained also on harm reduction. Obtained from model fit 0.56 95% C.I. (0.54-0.57). Since more interventions are introduces gradually with time, this rate is reduced even more after the harm reduction package was introduced (1988). Reducing the β_{h} exponentially after 1988 did not improved the fit.	Model fit

Supplementary Table 3. Mean time to HAART (in years) by disease stage as approximated by CD4 counts at baseline (for patients diagnosed before HAART introduction baseline was set to 1996), among IDUs in the Swiss HIV Cohort Study, by diagnosis period. The rates were estimated with parametric survival regression model using the Weibull distribution.

	<2000	**[2000,2005)**	**[2005,2010)**	**[2010,2016]**
Recent (CD4 > 500)	7.6	3.5	3.3	1.1
Chronic (CD4 200-500)	4.1	1.9	1.8	0.6
AIDS (CD4 <200)	1.8	0.8	0.8	0.3

Supplementary Figure 5. Reported number of IDU in methadone treatment 1980-1987 and the reduced model (See Supplementary Table 2, parameter h) fit, used to estimate the rate of recruitment to restricted methadone (up to 1987). Data was obtained from: (25) and (7).



The force of infection was modelled as:

$$\lambda = \frac{\beta(t) \cdot \left(\sum_{i=\{R,C,A\}} I_{u,i} + \sum_{i=\{R,C,A\}} d_s \cdot I_{d,i}\right) + \beta_h(t) \cdot \left(\sum_{i=\{R,C,A\}} I_{u,h,i} + \sum_{i=\{R,C,A\}} (d_s/2) \cdot I_{d,h,i}\right)}{N\ of\ active\ injectors}$$

I_u – infected injectors -undiagnosed, I_d – infected injectors- diagnosed
R – recent stage, C – chronic stage, A – AIDS stage
d_s – fraction of HIV diagnosed that still share injection paraphernalia (see Supplementary Table 2)
h- on harm reduction package

Model equations

See Supplementary Table 4 for initial values and interpretation of the compartments.

Non-injectors

$$\frac{dNv}{dt} = b(t)\cdot(1-f\_iv) + o \cdot Nvh - h(t) \cdot Nv - v(t) \cdot Nv - Nv \cdot (m\_n+w)$$

$$\frac{dNvh}{dt} = -o \cdot Nvh + h(t) \cdot Nv - v(t) \cdot Nvh - Nvh \cdot (m\_n\_h+w\_h)$$

Susceptible

$$\frac{dS}{dt} = b(t) \cdot f\_iv + v(t) \cdot Nv + o \cdot Sh - h(t) \cdot S - \lambda \cdot S - S \cdot (m\_s\_i+w)$$

$$\frac{dSh}{dt} = v(t) \cdot Nvh - o \cdot Sh + h(t) \cdot S - \lambda \cdot Sh - Sh \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot Sh$$

Infected undiagnosed

$$\frac{dI\_u\_R}{dt} = \lambda \cdot S + o \cdot I\_u\_Rh - h(t) \cdot I\_u\_R - r\_c \cdot I\_u\_R - a(t) \cdot I\_u\_R - I\_u\_R \cdot (m\_s\_i+w)$$

$$\frac{dI\_u\_Rh}{dt} = \lambda \cdot Sh - o \cdot I\_u\_Rh + h(t) \cdot I\_u\_R - r\_c \cdot I\_u\_Rh - a\_h(t) \cdot I\_u\_Rh - I\_u\_Rh \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot I\_u\_Rh$$

$$\frac{dI\_u\_C}{dt} = r\_c \cdot I\_u\_R + o \cdot I\_u\_Ch - h(t) \cdot I\_u\_C - c\_a \cdot I\_u\_C - a(t) \cdot I\_u\_C - I\_u\_C \cdot (m\_s\_i+w)$$

$$\frac{dI\_u\_Ch}{dt} = r\_c \cdot I\_u\_Rh - o \cdot I\_u\_Ch + h(t) \cdot I\_u\_C - c\_a \cdot I\_u\_Ch - a\_h(t) \cdot I\_u\_Ch - I\_u\_Ch \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot I\_u\_Ch$$

$$\frac{dI\_u\_A}{dt} = c\_a \cdot I\_u\_C + o \cdot I\_u\_Ah - h(t) \cdot I\_u\_A - a(t) \cdot I\_u\_A - I\_u\_A \cdot (m\_a + w)$$

$$\frac{dI\_u\_Ah}{dt} = c\_a \cdot I\_u\_Ch - o \cdot I\_u\_Ah + h(t) \cdot I\_u\_A - a\_h(t) \cdot I\_u\_Ah - I\_u\_Ah \cdot (m\_a + w\_h) - fr\_iv \cdot I\_u\_Ah$$

Diagnosed

$$\frac{dI\_d\_R}{dt} = a(t) \cdot I\_u\_R + r \cdot Tr\_R + o \cdot I\_d\_Rh - h\_hivp(t) \cdot I\_d\_R - r\_c \cdot I\_d\_R - g\_R(t) \cdot I\_d\_R - I\_d\_R \cdot (m\_s\_i+w)$$

$$\frac{dI\_d\_Rh}{dt} = a\_h(t) \cdot I\_u\_Rh + r\_h \cdot Tr\_Rh - o \cdot I\_d\_Rh + h\_hivp(t) \cdot I\_d\_R - r\_c \cdot I\_d\_Rh - g\_R\_h(t) \cdot I\_d\_Rh - I\_d\_Rh \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot I\_d\_Rh$$

$$\frac{dI\_d\_C}{dt} = r\_c \cdot I\_d\_R + a(t) \cdot I\_u\_C + r \cdot Tr\_C + o \cdot I\_d\_Ch - h\_hivp(t) \cdot I\_d\_C - c\_a \cdot I\_d\_C - g\_C(t) \cdot I\_d\_C - I\_d\_C \cdot (m\_s\_i+w)$$

$$\frac{dI\_d\_Ch}{dt} = r\_c \cdot I\_d\_Rh + a\_h(t) \cdot I\_u\_Ch + r\_h \cdot Tr\_Ch - o \cdot I\_d\_Ch + h\_hivp(t) \cdot I\_d\_C - c\_a \cdot I\_d\_Ch - g\_C\_h(t) \cdot I\_d\_Ch - I\_d\_Ch \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot I\_d\_Ch$$

$$\frac{dI\_d\_A}{dt} = c\_a \cdot I\_d\_C + a(t) \cdot I\_u\_A + r \cdot Tr\_A + o \cdot I\_d\_Ah - h\_hivp(t) \cdot I\_d\_A - g\_A(t) \cdot I\_d\_A - I\_d\_A \cdot (m\_a +w)$$

$$\frac{dI\_d\_Ah}{dt} = c\_a \cdot I\_d\_Ch + a\_h(t) \cdot I\_u\_Ah + r\_h \cdot Tr\_Ah - o \cdot I\_d\_Ah + h\_hivp(t) \cdot I\_d\_A - g\_A\_h(t) \cdot I\_d\_Ah - I\_d\_Ah \cdot (m\_a +w\_h) - fr\_iv \cdot I\_d\_Ah$$

Treated

$$\frac{dTr\_R}{dt} = g\_R(t) \cdot I\_d\_R + o \cdot Tr\_Rh - h\_hivp(t) \cdot Tr\_R - r \cdot Tr\_R - Tr\_R \cdot (m\_s\_i+w)$$

$$\frac{dTr\_Rh}{dt} = g\_R\_h(t) \cdot I\_d\_Rh - o \cdot Tr\_Rh + h\_hivp(t) \cdot Tr\_R - r\_h \cdot Tr\_Rh - Tr\_Rh \cdot (m\_s\_i\_h+w\_h) - fr\_iv \cdot Tr\_Rh$$

$$\frac{dTr\_C}{dt} = g\_C(t) \cdot I\_d\_C + o \cdot Tr\_Ch - h\_hivp(t) \cdot Tr\_C - r \cdot Tr\_C - Tr\_C \cdot (m\_s\_i+w)$$

$$\frac{dTr\_Ch}{dt} = g\_C\_h(t) \cdot I\_d\_Ch - o \cdot Tr\_Ch + h\_hivp(t) \cdot Tr\_C - r\_h \cdot Tr\_Ch - Tr\_Ch \cdot (m\_s\_i\_h+w\_h)-fr\_iv \cdot Tr\_Ch$$

$$\frac{dTr\_A}{dt} = g\_A(t) \cdot I\_d\_A + o \cdot Tr\_Ah - h\_hivp(t) \cdot Tr\_A - r \cdot Tr\_A - Tr\_A \cdot (m\_at+ w)$$

$$\frac{dTr\_Ah}{dt} = g\_A\_h(t) \cdot I\_d\_Ah - o \cdot Tr\_Ah + h\_hivp(t) \cdot Tr\_A - r\_h \cdot Tr\_Ah - Tr\_Ah \cdot (m\_at+ w\_h)-fr\_iv \cdot Tr\_Ah$$

Past injectors

$$\frac{dPv\_S}{dt} = fr\_iv \cdot Sh - (m\_s\_i\_h+w\_h) \cdot Pv\_S$$

inflow from undiagnosed

$$\frac{dPv\_IuR}{dt} = fr\_iv \cdot I\_u\_Rh - r\_c \cdot Pv\_IuR - a\_h(t) \cdot Pv\_IuR - (m\_s\_i\_h+w\_h) \cdot Pv\_IuR$$

$$\frac{dPv\_IuC}{dt} = fr\_iv \cdot I\_u\_Ch + r\_c \cdot Pv\_IuR - c\_a \cdot Pv\_IuC - a\_h(t) \cdot Pv\_IuC - (m\_s\_i\_h+w\_h) \cdot Pv\_IuC$$

$$\frac{dPv\_IuA}{dt} = fr\_iv \cdot I\_u\_Ah + c\_a \cdot Pv\_IuC - a\_h(t) \cdot Pv\_IuA - (m\_a +w\_h) \cdot Pv\_IuA$$

inflow from diagnosed

$$\frac{dPv\_IdR}{dt} = fr\_iv \cdot I\_d\_Rh + a\_h(t) \cdot Pv\_IuR + r\_h \cdot Pv\_T\_R - r\_c \cdot Pv\_IdR - g\_R\_h(t) \cdot Pv\_IdR - (m\_s\_i\_h+w\_h) \cdot Pv\_IdR$$

$$\frac{dPv\_IdC}{dt} = fr\_iv \cdot I\_d\_Ch + a\_h(t) \cdot Pv\_IuC + r\_c \cdot Pv\_IdR + r\_h \cdot Pv\_T\_C - c\_a \cdot Pv\_IdC - g\_C\_h(t) \cdot Pv\_IdC - (m\_s\_i\_h+w\_h) \cdot Pv\_IdC$$

$$\frac{dPv\_IdA}{dt} = fr\_iv \cdot I\_d\_Ah + c\_a \cdot Pv\_IdC + r\_h \cdot Pv\_T\_A + a\_h(t) \cdot Pv\_IuA - g\_A\_h(t) \cdot Pv\_IdA - (m\_a +w\_h) \cdot Pv\_IdA$$

inflow from Treated

$$\frac{dPv\_T\_R}{dt} = fr\_iv \cdot Tr\_Rh + g\_R\_h(t) \cdot Pv\_IdR - r\_h \cdot Pv\_T\_R - Pv\_T\_R \cdot (m\_s\_i\_h + w\_h)$$

$$\frac{dPv\_T\_C}{dt} = fr\_iv \cdot Tr\_Ch + g\_C\_h(t) \cdot Pv\_IdC - r\_h \cdot Pv\_T\_C - Pv\_T\_C \cdot (m\_s\_i\_h + w\_h)$$

$$\frac{dPv\_T\_A}{dt} = fr\_iv \cdot Tr\_Ah + g\_A\_h(t) \cdot Pv\_IdA - r\_h \cdot Pv\_T\_A - Pv\_T\_A \cdot (m\_at+ w\_h)$$

Not part of the ODE system: Counters for the output

incidence - new diagnosed HIV

HIV_new_diagnosed= (a(t) · I_u_R + a(t) · I_u_C + a(t) · I_u_A + a_h(t) · I_u_Rh + a_h(t) · I_u_Ch +a_h(t) · I_u_Ah + a_h(t) · Pv_IuR + a_h(t) · Pv_IuC +a_h(t) · Pv_IuA)

new reported HIV deaths

Reported_AIDS_death= (I_d_A · m_a + m_at · Tr_A + I_d_Ah · m_a +Tr_Ah · m_at + m_a · Pv_IdA + Pv_T_A · m_at)

Supplementary Table 4. Initial values at 1980 (t=0) and interpretation of the compartments.

Compartment	Interpretation	Initial value
Nv	Non-injectors, not on harm reduction	250
Nvh	Non-injectors, on harm reduction	0
S	Injectors, Susceptible, not on harm reduction	3099
Sh	Injectors, Susceptible, on harm reduction	650
I_u_R	Injectors, Infected, recent stage, undiagnosed, not on harm reduction	**1 – index case**
I_u_C	Injectors, Infected, chronic stage, undiagnosed, not on harm reduction	0
I_u_A	Injectors, Infected, AIDS stage, undiagnosed, not on harm reduction	0
I_u_Rh	Injectors, Infected, recent stage, undiagnosed, on harm reduction	0
I_u_Ch	Injectors, Infected, chronic stage, undiagnosed, on harm reduction	0
I_u_Ah	Injectors, Infected, AIDS stage, undiagnosed, on harm reduction	0
I_d_R	Injectors, Infected, recent stage, diagnosed, not on harm reduction	0
I_d_C	Injectors, Infected, chronic stage, diagnosed, not on harm reduction	0
I_d_A	Injectors, Infected, AIDS stage, diagnosed, not on harm reduction	0
I_d_Rh	Injectors, Infected, recent stage, diagnosed, on harm reduction	0
I_d_Ch	Injectors, Infected, chronic stage, diagnosed, on harm reduction	0
I_d_Ah	Injectors, Infected, AIDS stage, diagnosed, on harm reduction	0
Tr_R	Injectors, Treated, recent stage, not on harm reduction	0
Tr_C	Injectors, Treated, chronic stage, not on harm reduction	0
Tr_A	Injectors, Treated, AIDS stage, not on harm reduction	0
Tr_Rh	Injectors, Treated, recent stage, on harm reduction	0
Tr_Ch	Injectors, Treated, chronic stage, on harm reduction	0
Tr_Ah	Injectors, Treated, AIDS stage, on harm reduction	0
Pv_S	Past-injectors, Susceptible	0
Pv_IuR	Past-injectors, Infected, recent stage, undiagnosed, on harm reduction	0
Pv_IuC	Past-injectors, Infected, chronic stage, undiagnosed, on harm reduction	0
Pv_IuA	Past-injectors, Infected, AIDS stage, undiagnosed, on harm reduction	0

Pv_IdR	Past-injectors, Infected, recent stage, diagnosed, on harm reduction	0
Pv_IdC	Past-injectors, Infected, chronic stage, diagnosed, on harm reduction	0
Pv_IdA	Past-injectors, Infected, AIDS stage, diagnosed, on harm reduction	0
Pv_T_R	Past-injectors, Treated, recent stage, on harm reduction	0
Pv_T_C	Past-injectors, Treated, chronic stage, on harm reduction	0
Pv_T_A	Past-injectors, Treated, AIDS stage, on harm reduction	0

Model fitting and sensitivity range

The model was fitted to the annual numbers of HIV diagnosed and AIDS deaths using negative log-likelihood-distributed error (see below), with the R function “optim” and the "L-BFGS-B" method. The optimization converged. Supplementary Table 5 shows the parameters that were obtained from model fit. Confidence intervals were obtained from the Hessian, that was calculated with the package “numDeriv” (version 2016.8-1).

The sensitivity range was obtained by sampling 10,000 times from the parameter space within the confidence intervals using the uniform distribution. Next, for each combination, the model was simulated and the sum of the difference between the HIV incidence and AIDS deaths in the best-fit model and each random combination was calculated. The highest score represents the parameters combination that provides the highest deviation below the best-fit while the lowest score provides the highest deviation from above (See Figure 3 in the main manuscript). These two parameter sets were utilized to obtain the model sensitivity range (Supplementary Table 5). We opted for this procedure to deal with relatively narrow confidence intervals obtained from the Hessian.

Supplementary Table 5. Parameters that were obtained by model fitting and parameters combination used for sensitivity analysis.

	Initial guess	Lower bound	Upper bound	Best fit	Lower 95% C.I	Upper 95% C.I	Lower sensitivity scenario	Upper sensitivity scenario
$\boldsymbol{\beta_r}$	0.25	0.05	0.6	0.548	0.421	0.675	0.674	0.434
h	2	0.5	3	1.027	0.672	1.381	1.374	0.673
$\boldsymbol{\beta_0}$	1.4	1	2	1.301	1.28	1.322	1.28	1.321
φ	0.8	0.01	0.9	0.568	0.524	0.613	0.53	0.608

Meaning of the parameters: $\boldsymbol{\beta_r}$ Rate of decline of non-harm reduction transmission rate after introduction of harm reduction package, **h** Annual rate of recruitment to any harm reduction after the introduction of harm reduction package, $\boldsymbol{\beta_0}$ Initial epidemic transmission coefficient, **φ** The fraction of the initial non-harm reduction transmission coefficient ($\boldsymbol{\beta_0}$) that remained intact also on harm reduction (because harm reduction is not 100% effective in preventing HIV).

Phylogenetic analysis to quantify the epidemic spillover to the general population

All clusters that were comprised only of Swiss sequences and had at least one IDU and one heterosexual individual were extracted. For each IDU, the nodes were traversed back until the cluster either contained another IDU individual or a risk group that is other than IDU or HET, then the largest previous cluster was returned (Supplementary Figure 4). Because of a very large prevalence gradient – as described in (30) - of 20%-10% prevalence among IDU and less than 0.5% prevalence among heterosexuals, the direction of transmission in a cluster was always assumed to be from IDU to heterosexuals. The number of heterosexuals that would have been infected in a scenario without harm reduction was calculated as:

$$\left(\frac{N\ of\ heterosexuals\ in\ the\ extracted\ clusters}{\text{N of IDU on the tree}}\right) \cdot \text{(Incidence in the scenario without harm reduction - Incidence in the best fit)}$$

$$\left(\frac{499}{2399}\right) \cdot 15{,}903 = 3{,}308$$

An alternative way that uses all the IDU in the cohort in the denominator and accounts for sparse sampling of heterosexuals on the tree by a weighting factor w=0.65, produces a more conservative result (that is we reported in the main manuscript):

$$\left(\frac{\text{N of heterosexuals in the extracted clusters}}{\text{N of IDU in the cohort}}\right) \cdot \frac{1}{w} \cdot \text{(Incidence in the scenario without harm reduction - Incidence in the best fit)}$$

$$\left(\frac{499}{4806}\right) \cdot \frac{1}{0.65} \cdot 15{,}903 = 2{,}540$$

Supplementary Figure 6. Vending machines that offer sterile injection paraphernalia and condoms in Zürich, Switzerland. Left: new model (2017), Right: old model. The vending automates offer a package that contains: 2 x 1 ml syringes with filter, 2 needles (0.45 x 12 mm), 2 x 0.5 g Ascorbin, 2 x 1,5 ml NaCl, 2 x Alcohol pads, 2 x dry pads. Another option is available for a set of larger (0.5 x 16 mm) needles. A pack of six condoms is also offered. Price for all items: 2 Swiss Francs (~2 US dollars, as for May 2017). Photos: Alex Marzel.

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Supplementary Material: Chapter V *"High rates of subsequent asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection"*

Supplementary Table 1. Detected STIs by anatomical site.

	Chlamydia	Gonorrhea
Pharyngeal	5 (15.6%)	4 (26.7%)
Rectal	20 (62.5%)	8 (53.3%)
Genital	7 (21.9%)	3 (20%)
Non-missing/Total	32/40 (80%)	15/20 (75%)

Supplementary Table 2. Association of selected factors with incident STI.

	Overall	No incident STI	Incident STI	p-value	p-value (adjusted) [§]
Number of patients	73	52	21		
Age at diagnosis (median [IQR])	33.1 [28.2, 40.3]	32.8 [28.1, 40.9]	34.7 [29.3, 37.2]	0.724	1
Sex , Male (%)	72 (98.6)	51 (98.1)	21 (100.0)	1	1
Years since diagnosis (median [IQR])	5.4 [3.3, 8.9]	6.2 [3.8, 9.1]	3.4 [3.0, 6.1]	0.039	0.184
Diagnosis year (median [IQR])	2010.0 [2007.0, 2012.0]	2009.0 [2007.0, 2012.0]	2012.0 [2010.0, 2013.0]	0.053	0.189
Ethnicity , White (%)	70 (95.9)	50 (96.2)	20 (95.2)	1	1
Higher education , Yes (%)	20 (27.4)	15 (28.8)	5 (23.8)	0.777	1
Risk group , MSM (%)	68 (93.2)	47 (90.4)	21 (100.0)	0.313	0.729
Stable partner , Yes (%)	36 (49.3)	27 (51.9)	9 (42.9)	0.607	1
Sexual contact , Yes (%)	72 (98.6)	51 (98.1)	21 (100.0)	1	1
Anal intercourse (%)				0.964	1
No	6 (8.2)	5 (9.6)	1 (4.8)		

Receptive	8 (11.0)	6 (11.5)	2 (9.5)		
Insertive	6 (8.2)	4 (7.7)	2 (9.5)		
Both	53 (72.6)	37 (71.2)	16 (76.2)		
Oral sex only , Yes (%)	1 (1.4)	1 (1.9)	0 (0.0)	1	1
Number of sexual partners (median [IQR])	2.0 [1.0, 5.0]	2.0 [1.0, 3.2]	3.0 [2.0, 6.0]	0.004	0.041
Condomless sex , Yes (%)	37 (50.7)	25 (48.1)	12 (57.1)	0.607	1
STI symptoms , Yes (%)	13 (17.8)	5 (9.6)	8 (38.1)	0.007	0.05
Number of symptoms (median [IQR])	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 1.0]	0.003	0.041
CD4 at STI screen (median [IQR])	734.0 [544.0, 887.0]	752.0 [540.8, 893.2]	715.0 [579.0, 876.0]	0.55	1
CD4/CD8 ratio (median [IQR])	1.2 [0.9, 1.6]	1.2 [0.9, 1.6]	1.1 [0.9, 1.6]	0.559	1
Virally suppressed , Yes (%)	68 (93.2)	49 (94.2)	19 (90.5)	0.621	1
History of Syphilis , Yes (%)	25 (34.2)	15 (28.8)	10 (47.6)	0.174	0.487
History of depression , Yes (%)	25 (34.2)	18 (34.6)	7 (33.3)	1	1

Psychiatric history , Yes (%)	21 (28.8)	16 (30.8)	5 (23.8)	0.776	1
Ever smoked , Yes (%)	37 (51.4)	27 (52.9)	10 (47.6)	0.797	1
Any recent drug use , Yes (%)	11 (15.1)	3 (5.8)	8 (38.1)	0.001	0.039
Binge drinking (%)				0.054	0.189
Never	56 (80.0)	43 (86.0)	13 (65.0)		
Monthly or less	8 (11.4)	3 (6.0)	5 (25.0)		
Weekly or more	6 (8.6)	4 (8.0)	2 (10.0)		
Housing (%)				0.261	0.663
Alone	57 (83.8)	44 (86.3)	13 (76.5)		
Friends	9 (13.2)	5 (9.8)	4 (23.5)		
Other	2 (2.9)	2 (3.9)	0 (0.0)		
Traveled to tropics , Yes (%)	9 (12.3)	7 (13.5)	2 (9.5)	1	1
Physical activity (%)				0.113	0.351
Low	19 (26.0)	17 (32.7)	2 (9.5)		
Moderate	33 (45.2)	21 (40.4)	12 (57.1)		
High	21 (28.8)	14 (26.9)	7 (33.3)		
BMI (median [IQR])	23.5 [21.8,	24.7 [22.6, 27.0]	22.7 [21.6, 23.4]	0.018	0.101

25.7]

[§] **- Benjamini-Hochberg adjustment**

Supplementary 3. Risk behaviour questionnaire

Patient ID

Date: I__I__I__I__I__I__I__I__I

STI-screening is offered to all participants every three months. STI-testing is done by PCR test for chlamydia and gonorrhea from the rectum, pharynx, and the urethra in a pooled analysis, and for syphilis by serology, and for hepatitis C virus in case of elevated liver enzymes by anti-HCV-antibodies.

All questions refer to the last 3 months prior to the STI-screening:

- Sexual contacts? yes ☐ no ☐

- Closed partnership? yes ☐ no ☐

- Condom use? With exclusive partner yes ☐ no ☐

  With occasional partner yes ☐ no ☐

- Mode of sexual contact: ☐ oral sex

  ☐ anal intercourse insertive☐ receptive☐

  ☐ vaginal intercourse

- Number of sex partners: _____

- Symptoms/signs of STI? yes ☐ no ☐

- Throat: ☐Redness

☐Pain

☐Ulcera/lesions

☐Enlarged lymph nodes in the neck region

- Vagina/Penis: ☐Redness

☐Pain

☐Ulcera/lesions

☐Discharge

☐Enlarged lymph nodes in the inguinal region

- Anal: ☐Redness

☐Pain

☐Discharge Ulcera/lesions

☐Ulcera/lesions

The specimens can be obtained self-collected by the participant, or by the physician

Supplementary 4. Diagnosis of STI

Swabs obtained from the urethra, rectum and pharynx were tested by polymerase chain reaction for Chlamydia trachomatis and Neisseria gonorrhoeae (COBAS TaqMan 4800 CT Test v2.0, Roche Diagnostics, Rotkreuz, Switzerland); tests that were positive for C. trachomatis were further examined and typed for Lymphogranuloma venereum (LGV) strains (inhouse duplex real-time rapid PCR C. trachomatis variant L1-L3 and L2b-specific PCR, Institute for Clinical Microbiology, University of Basel Switzerland). For the pooled screening, a combined specimen was obtained with a single swab first from the pharynx, followed by the same swab from the rectum, and an additional swab from the urethra. All swabs were then put together in a single tube for the pooled PCR testing. Patients with a diagnosed STI were informed by phone from the treating physician and received antibiotic treatment, where appropriate, at the outpatient clinic.

The non-treponemal test contained either Venereal Diseases Research Laboratory (VDRL) or Rapid plasma reagin (RPR) titers; and the treponemal branch included TPPA. Blood was tested for syphilis by TPPA (SERODIA®-TPPA, Fujirebio, Tokyo, Japan). If TPPA yielded a positive result, testing with VDRL and/or RPR was added. Patients with both tests negative and seroconversion of both tests were considered as incident cases. Positive TPPA and increasing VDRL/RPR by 4-fold were also considered as incident cases (reinfection). Positive TPPA and negative VDRL/RPR were considered as previously positive tests. For positive cases, a history of sexual exposure and a clinical exam was performed. VDRL/RPR positive and TPPA negative were repeated and if TPPA remained negative, considered to be false positive cases (however, there was none of this cases in our study).

Patients were also tested yearly for anti HCV IgG (ARCHITECT Anti-HCV assay, Abbott, Wiesbaden, Germany). In patients presenting with elevated transaminases, considering >50 U/l as the

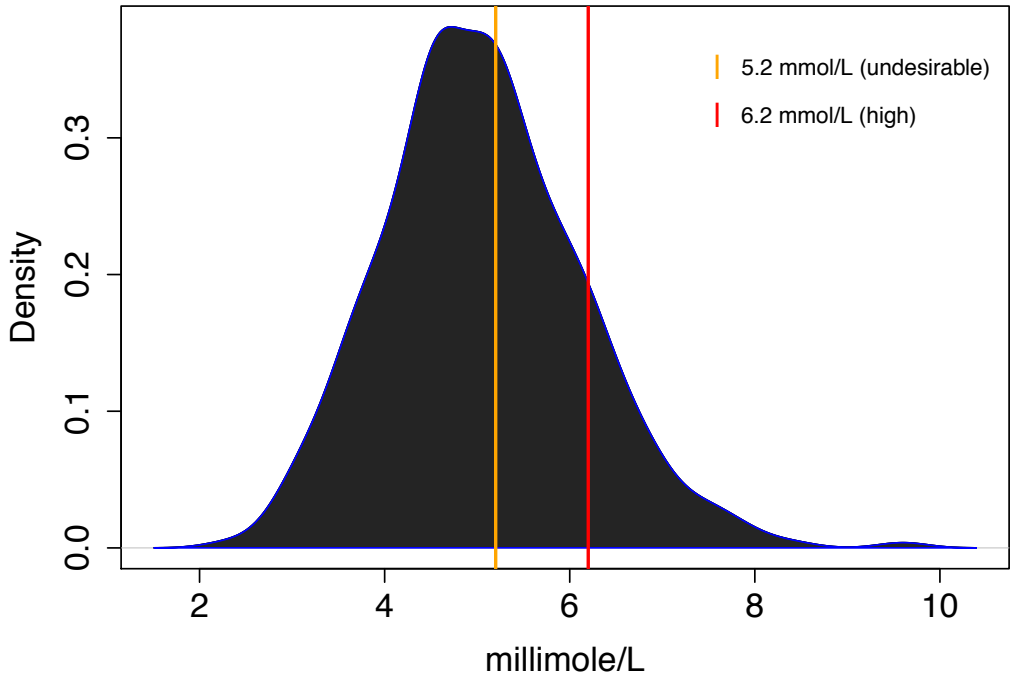
upper limit of a normal value, blood was additionally tested for Hepatitis C by PCR (COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0, analytical sensitivity 15 IU/mL).

Supplementary 5. Treatment of STI

Patients with a diagnosed STI were informed by phone from treating physician and received antibiotic treatment, where appropriate, at the outpatient clinic. Uncomplicated chlamydial infection was treated with azithromycin 1 g as a single dose or doxycycline 100 mg twice daily for seven days, LGV doxycycline 100 mg twice daily for 21 days or azithromycin 1 g as a single dose repeated every week for three weeks. Gonorrhea was treated with ceftriaxone 250 mg by intramuscular injection in combination with azithromycin 1 g as a single dose orally. Early syphilis without signs of central nervous system involvement was treated with a single dose of 2.4 million unit's long-acting benzathine penicillin G (BPG) by intramuscular injection. Partner management was discussed at the same visit and concomitant sex partners were informed by the patient if possible.

Supplementary Material: Chapter VI **"***Dietary patterns and physical activity correlate with total cholesterol independently of lipid lowering drugs and ART in aging HIV positive individuals"*

Supplementary Figure 1. Distribution of total cholesterol values (as Kernel Density).



Supplementary Table 1. Demographic and clinical covariates by quartiles of dietary pattern I (meat, refined/milled grains, carbonated beverages, coffee).

	Quartile I	**Quartile II**	**Quartile III**	**Quartile IV**	**P**
	n=103	n= 97	n=99	n=96	
Age (median [IQR])	56.8 [54.0, 62.3]	55.0 [51.6, 59.7]	54.8 [52.1, 60.3]	55.2 [51.8, 59.8]	0.055
Sex, female (%)	26 (25.2)	11 (11.3)	11 (11.1)	9 (9.4)	0.004
Ethnicity (%)					0.126
White	94 (91.3)	92 (94.8)	94 (94.9)	93 (96.9)	
Black	8 (7.8)	4 (4.1)	3 (3.0)	0 (0.0)	
Hispanic	1 (1.0)	1 (1.0)	2 (2.0)	3 (3.1)	
Risk group (%)					0.022
Heterosexual	38 (36.9)	20 (20.6)	20 (20.2)	30 (31.2)	
Men-who-have-Sex-with-Men	45 (43.7)	68 (70.1)	65 (65.7)	55 (57.3)	
Injecting drug users	9 (8.7)	4 (4.1)	5 (5.1)	6 (6.2)	
Other	11 (10.7)	5 (5.2)	9 (9.1)	5 (5.2)	
University education, yes (%)	15 (14.6)	11 (11.3)	16 (16.2)	13 (13.5)	0.801
Dwelling alone ,yes (%)	41 (39.8)	45 (46.4)	41 (41.4)	35 (36.5)	0.562
Current smoking, yes (%)	22 (21.4)	29 (29.9)	31 (31.3)	44 (45.8)	0.003
BMI (%)					0.745
Normal (>=18.5 - 25)	53 (51.5)	51 (52.6)	52 (52.5)	56 (58.3)	
Overweight (>=25 - 30)	37 (35.9)	34 (35.1)	32 (32.3)	25 (26.0)	
Obese (>=30)	9 (8.7)	10 (10.3)	14 (14.1)	13 (13.5)	
Underweight (<18.5)	4 (3.9)	2 (2.1)	1 (1.0)	2 (2.1)	
Depression, yes (%)	19 (18.4)	13 (13.4)	14 (14.1)	13 (13.5)	0.711
Diabetes, yes (%)	7 (6.8)	5 (5.2)	4 (4.0)	7 (7.3)	0.751
Hypertension, yes (%)	44 (42.7)	29 (29.9)	33 (33.3)	29 (30.2)	0.184
Lipid lowering drugs, yes (%)	22 (21.4)	28 (28.9)	17 (17.2)	31 (32.3)	0.058
Virally suppressed, yes (%)	100 (97.1)	91 (93.8)	97 (98.0)	91 (94.8)	0.413
CD4 (median [IQR])	657.0 [536.5, 862.0]	660.0 [503.0, 803.0]	624.0 [454.0, 804.5]	628.5 [488.8, 805.0]	0.814
Years on ART (median [IQR])	13.9 [8.7, 20.5]	14.9 [8.0, 20.2]	14.2 [9.0, 20.5]	14.3 [8.9, 20.4]	0.979
On NRTI, yes (%)	91 (88.3)	76 (78.4)	70 (70.7)	83 (86.5)	0.005
On NTRTI, yes (%)	46 (44.7)	40 (41.2)	44 (44.4)	62 (64.6)	0.004
On NNRTI, yes (%)	27 (26.2)	29 (29.9)	30 (30.3)	31 (32.3)	0.819
On PI, yes (%)	23 (22.3)	24 (24.7)	23 (23.2)	37 (38.5)	0.036
On INTI, yes (%)	61 (59.2)	54 (55.7)	60 (60.6)	52 (54.2)	0.781
Physical activity (%)					0.718
Never	30 (29.1)	32 (33.0)	28 (28.3)	34 (35.4)	
1-4 times a month	8 (7.8)	10 (10.3)	13 (13.1)	9 (9.4)	
1-2 times a week	26 (25.2)	18 (18.6)	23 (23.2)	14 (14.6)	
>=3 times a week	39 (37.9)	37 (38.1)	35 (35.4)	39 (40.6)	
Dietary change in the last two years (%)					0.291

	Quartile I	Quartile II	Quartile III	Quartile IV	P
No	89 (86.4)	92 (94.8)	93 (93.9)	86 (89.6)	
Became vegetarian	2 (1.9)	0 (0.0)	0 (0.0)	1 (1.0)	
Other change	12 (11.7)	5 (5.2)	6 (6.1)	9 (9.4)	

P-values represents chi-square test for categorical variables, ANOVA for numeric normal and Kruskal-Wallis Rank Sum Test for non-normally distributed.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; INTI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NTRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Supplementary Table 2. Demographic and clinical covariates by quartiles of dietary pattern II (organ meats, poultry, fish/seafood, alcohol).

	Quartile I	Quartile II	Quartile III	Quartile IV	p
	n=101	n=99	n=98	n= 97	
Age (median [IQR])	56.2 [52.9, 63.2]	56.2 [53.0, 60.3]	54.3 [51.3, 59.7]	55.3 [51.8, 59.9]	0.123
Sex, female (%)	27 (26.7)	13 (13.1)	9 (9.2)	8 (8.2)	0.001
Ethnicity (%)					0.096
White	101 (100.0)	94 (94.9)	90 (91.8)	88 (90.7)	
Black	0 (0.0)	3 (3.0)	5 (5.1)	7 (7.2)	
Hispanic	0 (0.0)	2 (2.0)	3 (3.1)	2 (2.1)	
Risk group (%)					0.129
Heterosexual	38 (37.6)	23 (23.2)	21 (21.4)	26 (26.8)	
Men-who-have-Sex-with-Men	45 (44.6)	63 (63.6)	64 (65.3)	61 (62.9)	
Injecting drug users	8 (7.9)	7 (7.1)	6 (6.1)	3 (3.1)	
Other	10 (9.9)	6 (6.1)	7 (7.1)	7 (7.2)	
University education, yes (%)	11 (10.9)	8 (8.1)	20 (20.4)	16 (16.5)	0.056
Dwelling alone ,yes (%)	52 (51.5)	41 (41.4)	37 (37.8)	32 (33.0)	0.055
Current smoking, yes (%)	36 (35.6)	28 (28.3)	25 (25.5)	37 (38.1)	0.185
BMI (%)					0.167
Normal (>=18.5 - 25)	60 (59.4)	53 (53.5)	50 (51.0)	49 (50.5)	
Overweight (>=25 - 30)	26 (25.7)	32 (32.3)	36 (36.7)	34 (35.1)	
Obese (>=30)	9 (8.9)	13 (13.1)	12 (12.2)	12 (12.4)	
Underweight (<18.5)	6 (5.9)	1 (1.0)	0 (0.0)	2 (2.1)	
Depression, yes (%)	20 (19.8)	16 (16.2)	11 (11.2)	12 (12.4)	0.313
Diabetes, yes (%)	7 (6.9)	3 (3.0)	8 (8.2)	5 (5.2)	0.442
Hypertension, yes (%)	37 (36.6)	29 (29.3)	38 (38.8)	31 (32.0)	0.484
Lipid lowering drugs, yes (%)	30 (29.7)	26 (26.3)	25 (25.5)	17 (17.5)	0.241
Virally suppressed, yes (%)	98 (97.0)	96 (97.0)	95 (96.9)	90 (92.8)	0.345
CD4 (median [IQR])	666.0 [538.0, 841.0]	653.0 [529.0, 796.0]	609.5 [473.8, 804.5]	615.0 [428.0, 817.0]	0.142
Years on ART (median [IQR])	15.9 [10.0, 20.5]	15.5 [9.0, 20.5]	12.8 [8.0, 19.8]	13.6 [8.4, 20.2]	0.255
On NRTI, yes (%)	86 (85.1)	72 (72.7)	79 (80.6)	83 (85.6)	0.077
On NTRTI, yes (%)	45 (44.6)	47 (47.5)	43 (43.9)	57 (58.8)	0.133
On NNRTI, yes (%)	33 (32.7)	31 (31.3)	28 (28.6)	25 (25.8)	0.722
On PI, yes (%)	33 (32.7)	28 (28.3)	20 (20.4)	26 (26.8)	0.274
On INTI, yes (%)	56 (55.4)	59 (59.6)	64 (65.3)	48 (49.5)	0.148
Physical activity (%)					0.003
Never	39 (38.6)	24 (24.2)	28 (28.6)	33 (34.0)	
1-4 times a month	13 (12.9)	12 (12.1)	3 (3.1)	12 (12.4)	
1-2 times a week	13 (12.9)	32 (32.3)	19 (19.4)	17 (17.5)	
>=3 times a week	36 (35.6)	31 (31.3)	48 (49.0)	35 (36.1)	

	Quartile I	Quartile II	Quartile III	Quartile IV	p
Dietary change in the last two years (%)					0.375
No	87 (86.1)	93 (93.9)	90 (91.8)	90 (92.8)	
Became vegetarian	2 (2.0)	0 (0.0)	1 (1.0)	0 (0.0)	
Other change	12 (11.9)	6 (6.1)	7 (7.1)	7 (7.2)	

P-values represents chi-square test for categorical variables, ANOVA for numeric normal and Kruskal-Wallis Rank Sum Test for non-normally distributed.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; INTI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NTRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Supplementary Table 3. Demographic and clinical covariates by quartiles of dietary pattern III (whole grains ,dairy products, eggs, leafy green vegetables ,other vegetables (raw and cooked), legumes/nuts/seeds, potatoes, boiled/mashed, pickled food, fruits, tea (black/green)).

	Quartile I	**Quartile II**	**Quartile III**	**Quartile IV**	**p**
n	99	100	97	99	
Age (median [IQR])	54.0 [51.7, 58.5]	56.6 [52.7, 61.2]	55.7 [52.3, 62.2]	56.6 [52.7, 61.9]	0.031
Sex, female (%)	12 (12.1)	15 (15.0)	16 (16.5)	14 (14.1)	0.851
Ethnicity (%)					0.549
White	96 (97.0)	91 (91.0)	93 (95.9)	93 (93.9)	
Black	2 (2.0)	7 (7.0)	2 (2.1)	4 (4.0)	
Hispanic	1 (1.0)	2 (2.0)	2 (2.1)	2 (2.0)	
Risk group (%)					0.555
Heterosexual	27 (27.3)	34 (34.0)	23 (23.7)	24 (24.2)	
Men-who-have-Sex-with-Men	56 (56.6)	53 (53.0)	62 (63.9)	62 (62.6)	
Injecting drug users	8 (8.1)	6 (6.0)	7 (7.2)	3 (3.0)	
Other	8 (8.1)	7 (7.0)	5 (5.2)	10 (10.1)	
University education, yes (%)	9 (9.1)	8 (8.0)	16 (16.5)	22 (22.2)	0.011
Dwelling alone ,yes (%)	45 (45.5)	41 (41.0)	36 (37.1)	40 (40.4)	0.698
Current smoking, yes (%)	45 (45.5)	33 (33.0)	28 (28.9)	20 (20.2)	0.002
BMI (%)					0.868
Normal (>=18.5 - 25)	58 (58.6)	55 (55.0)	47 (48.5)	52 (52.5)	
Overweight (>=25 - 30)	28 (28.3)	34 (34.0)	33 (34.0)	33 (33.3)	
Obese (>=30)	10 (10.1)	9 (9.0)	14 (14.4)	13 (13.1)	
Underweight (<18.5)	3 (3.0)	2 (2.0)	3 (3.1)	1 (1.0)	
Depression, yes (%)	14 (14.1)	7 (7.0)	23 (23.7)	15 (15.2)	0.012
Diabetes, yes (%)	5 (5.1)	7 (7.0)	5 (5.2)	6 (6.1)	0.93
Hypertension, yes (%)	29 (29.3)	40 (40.0)	30 (30.9)	36 (36.4)	0.359
Lipid lowering drugs, yes (%)	24 (24.2)	32 (32.0)	21 (21.6)	21 (21.2)	0.262
Virally suppressed, yes (%)	94 (94.9)	96 (96.0)	93 (95.9)	96 (97.0)	0.914
CD4 (median [IQR])	604.0 [458.0, 741.5]	632.5 [506.5, 837.5]	684.0 [534.0, 829.0]	617.0 [505.0, 810.0]	0.084
Years on ART (median [IQR])	14.0 [8.8, 20.4]	15.0 [9.1, 20.6]	14.9 [9.1, 20.2]	14.1 [7.3, 20.3]	0.698
On NRTI, yes (%)	79 (79.8)	78 (78.0)	79 (81.4)	84 (84.8)	0.65
On NTRTI, yes (%)	48 (48.5)	53 (53.0)	46 (47.4)	45 (45.5)	0.748
On NNRTI, yes (%)	27 (27.3)	31 (31.0)	27 (27.8)	32 (32.3)	0.838
On PI, yes (%)	25 (25.3)	29 (29.0)	28 (28.9)	25 (25.3)	0.878
On INTI, yes (%)	58 (58.6)	57 (57.0)	58 (59.8)	54 (54.5)	0.892
Physical activity (%)					0.172
Never	33 (33.3)	30 (30.0)	34 (35.1)	27 (27.3)	
1-4 times a month	14 (14.1)	5 (5.0)	13 (13.4)	8 (8.1)	
1-2 times a week	16 (16.2)	20 (20.0)	23 (23.7)	22 (22.2)	

	Quartile I	Quartile II	Quartile III	Quartile IV	p
>=3 times a week	36 (36.4)	45 (45.0)	27 (27.8)	42 (42.4)	
Dietary change in the last two years (%)					0.401
No	92 (92.9)	94 (94.0)	89 (91.8)	85 (85.9)	
Became vegetarian	1 (1.0)	1 (1.0)	0 (0.0)	1 (1.0)	
Other change	6 (6.1)	5 (5.0)	8 (8.2)	13 (13.1)	

P-values represents chi-square test for categorical variables, ANOVA for numeric normal and Kruskal-Wallis Rank Sum Test for non-normally distributed.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; INTI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NTRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Supplementary Table 4. Demographic and clinical covariates by quartiles of dietary pattern IV (pizza, deep fried foods, salty snacks, ice cream/pudding, desserts/sweet snacks, confectionary sugars/syrups, fruit juice/drinks).

	Quartile I	**Quartile II**	**Quartile III**	**Quartile IV**	**p**
	n=99	n=102	n=95	n=99	
Age (median [IQR])	56.5 [52.9, 64.3]	54.6 [52.0, 58.4]	56.1 [51.7, 59.5]	55.3 [51.8, 60.3]	0.069
Sex, female (%)	12 (12.1)	11 (10.8)	16 (16.8)	18 (18.2)	0.376
Ethnicity (%)					0.751
White	96 (97.0)	94 (92.2)	89 (93.7)	94 (94.9)	
Black	3 (3.0)	5 (4.9)	4 (4.2)	3 (3.0)	
Hispanic	0 (0.0)	3 (2.9)	2 (2.1)	2 (2.0)	
Risk group (%)					0.314
Heterosexual	32 (32.3)	26 (25.5)	26 (27.4)	24 (24.2)	
Men-who-have-Sex-with-Men	49 (49.5)	60 (58.8)	58 (61.1)	66 (66.7)	
Injecting drug users	6 (6.1)	9 (8.8)	6 (6.3)	3 (3.0)	
Other	12 (12.1)	7 (6.9)	5 (5.3)	6 (6.1)	
University education, yes (%)	7 (7.1)	14 (13.7)	22 (23.2)	12 (12.1)	0.012
Dwelling alone ,yes (%)	44 (44.4)	47 (46.1)	30 (31.6)	41 (41.4)	0.167
Current smoking, yes (%)	33 (33.3)	36 (35.3)	24 (25.3)	33 (33.3)	0.448
BMI (%)					0.468
Normal (>=18.5 - 25)	46 (46.5)	55 (53.9)	50 (52.6)	61 (61.6)	
Overweight (>=25 - 30)	40 (40.4)	32 (31.4)	34 (35.8)	22 (22.2)	
Obese (>=30)	11 (11.1)	13 (12.7)	9 (9.5)	13 (13.1)	
Underweight (<18.5)	2 (2.0)	2 (2.0)	2 (2.1)	3 (3.0)	
Depression, yes (%)	15 (15.2)	17 (16.7)	16 (16.8)	11 (11.1)	0.647
Diabetes, yes (%)	13 (13.1)	2 (2.0)	5 (5.3)	3 (3.0)	0.003
Hypertension, yes (%)	44 (44.4)	37 (36.3)	28 (29.5)	26 (26.3)	0.036
Lipid lowering drugs, yes (%)	33 (33.3)	29 (28.4)	19 (20.0)	17 (17.2)	0.031
Virally suppressed, yes (%)	95 (96.0)	98 (96.1)	93 (97.9)	93 (93.9)	0.581
CD4 (median [IQR])	651.0 [489.5, 874.5]	606.0 [484.2, 802.2]	647.0 [496.5, 771.0]	678.0 [511.5, 822.5]	0.572
Years on ART (median [IQR])	14.4 [9.3, 20.4]	13.8 [7.6, 20.3]	14.2 [9.0, 20.6]	15.5 [9.7, 20.2]	0.852
On NRTI, yes (%)	77 (77.8)	78 (76.5)	74 (77.9)	91 (91.9)	0.016
On NTRTI, yes (%)	46 (46.5)	51 (50.0)	45 (47.4)	50 (50.5)	0.927
On NNRTI, yes (%)	27 (27.3)	34 (33.3)	33 (34.7)	23 (23.2)	0.254
On PI, yes (%)	28 (28.3)	26 (25.5)	22 (23.2)	31 (31.3)	0.606
On INTI, yes (%)	54 (54.5)	62 (60.8)	50 (52.6)	61 (61.6)	0.492
Physical activity (%)					0.004
Never	38 (38.4)	43 (42.2)	17 (17.9)	26 (26.3)	
1-4 times a month	9 (9.1)	11 (10.8)	7 (7.4)	13 (13.1)	
1-2 times a week	20 (20.2)	18 (17.6)	28 (29.5)	15 (15.2)	
>=3 times a week	32 (32.3)	30 (29.4)	43 (45.3)	45 (45.5)	

	Quartile I	Quartile II	Quartile III	Quartile IV	p
Dietary change in the last two years (%)					0.8
No	89 (89.9)	91 (89.2)	89 (93.7)	91 (91.9)	
Became vegetarian	0 (0.0)	1 (1.0)	1 (1.1)	1 (1.0)	
Other change	10 (10.1)	10 (9.8)	5 (5.3)	7 (7.1)	

P-values represents chi-square test for categorical variables, ANOVA for numeric normal and Kruskal-Wallis Rank Sum Test for non-normally distributed.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; INTI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NTRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Supplementary Table 5. Factors correlating with total cholesterol, separately for patients on (n=98) and not on lipid lowering drugs (n=297) in two linear mixed-effect models with adjustment to the variables listed.

	Not on lipid lowering drugs				On lipid lowering drugs			
	Beta	**2.5 %**	**97.5 %**	**P value**	**Beta**	**2.5 %**	**97.5 %**	**P value**
Age	0.010	-0.009	0.028	0.314	0.004	-0.025	0.032	0.823
Female sex, vs. Male	0.574	0.198	0.949	0.004	0.258	-0.579	1.107	0.587
Risk group: MSM vs. HET	-0.061	-0.383	0.261	0.718	-0.247	-0.732	0.256	0.379
Risk group: IDU vs. HET	-0.398	-0.951	0.155	0.172	-1.148	-2.023	-0.278	0.022
Risk group: Other vs. HET	0.039	-0.401	0.480	0.865	-0.599	-1.825	0.592	0.381
On NRTI, yes vs. no	-0.016	-0.338	0.306	0.926	-0.327	-0.935	0.268	0.337
On NTRTI, yes vs. no	-0.236	-0.490	0.018	0.078	-0.546	-0.980	-0.076	0.037
On NNRTI, yes vs. no	0.171	-0.115	0.457	0.257	0.157	-0.280	0.626	0.540
On PI, yes vs. no	-0.078	-0.359	0.202	0.595	0.191	-0.243	0.669	0.464
On INTI, yes vs. no	0.080	-0.173	0.333	0.547	-0.182	-0.610	0.281	0.470
Physical activity 1-4 times a month vs. none	0.105	-0.297	0.507	0.619	-0.070	-0.839	0.760	0.878
Physical activity 1-2 times a week vs. none	0.019	-0.308	0.346	0.912	-0.160	-0.797	0.501	0.663
Physical activity >=3 times a week vs. none	-0.102	-0.382	0.178	0.488	-0.732	-1.166	-0.232	0.008
ln(dietary pattern I + 1)	0.234	0.011	0.457	0.047	0.352	-0.039	0.747	0.115
ln(dietary pattern II + 1)	0.055	-0.456	0.565	0.839	-0.159	-1.239	0.863	0.791
ln(dietary pattern III + 1)	0.054	-0.220	0.328	0.708	0.439	-0.168	0.996	0.186
ln(dietary pattern IV + 1)	-0.024	-0.323	0.275	0.878	-0.378	-0.904	0.111	0.189
fasting cholesterol, yes vs. no	-0.089	-0.406	0.227	0.592	0.113	-0.520	0.581	0.734

Abbreviations: Abbreviations: HET, heterosexual; IDU, injecting-drugs-users; INTI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NTRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.